Hormonal determinants of successful aging in men

The work presented in this thesis was conducted at the department of Internal Medicine of the Erasmus MC Rotterdam in close collaboration with the Julius Center for General Practice and Patient Oriented Research of the University Medical Center Utrecht and Andro Medical Research B.V..

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Hormonale determinanten van succesvol ouder worden bij mannen

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Aan mijn ouders

CONTENTS

LIST OF ABBREVIATIONS

ADL	Activities of Daily Living
BMD	Bone Mineral Density
BMI	Body Mass Index
β	Linear regression coefficient
Calc-FT	Calculated Free Testosterone
CRP	C-Reactive Protein
DHEA(S)	DeHydroEpiAndrostenedione (Sulfate)
E2	Estradiol
E1	Estrone
FT4	Free Thyronine
IGF-I	Insulin-like Growth Factor-1
IGFBP-1, -2, -3	Insulin-like Growth Factor Binding Protein-1, -2, -3
IGS	Isometric Grip Strength
IL-6	Interleukin-6
LH	Luteinizing Hormone
MaxLES	Maximum Leg Extensor Strength
MHAQ	Modified Health Assessment Questionnaire
Non-SHBG-T	Non-SHBG-Bound or bioavailable Testosterone
Non-SHBG-E2	Non-SHBG-Bound or bioavailable Estradiol
PPS	Physical Performance Score by Guralnik et al.
QLS	Quality of Life Satisfaction questionnaire
QoL	Quality of Life
r	Pearson correlation coefficient
rT3	Reverse T3
S.D.	Standard Deviation
S.E.	Standard Error
T3	Triiodothyronine
T4	Thyronine
T4S	T4 Sulfate
TBG	Thyroid Binding Globulin
TSH	Thyroid Stimulating Hormone
TT	Total Testosterone

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Chapter 2

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Chapter 3

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Chapter 6

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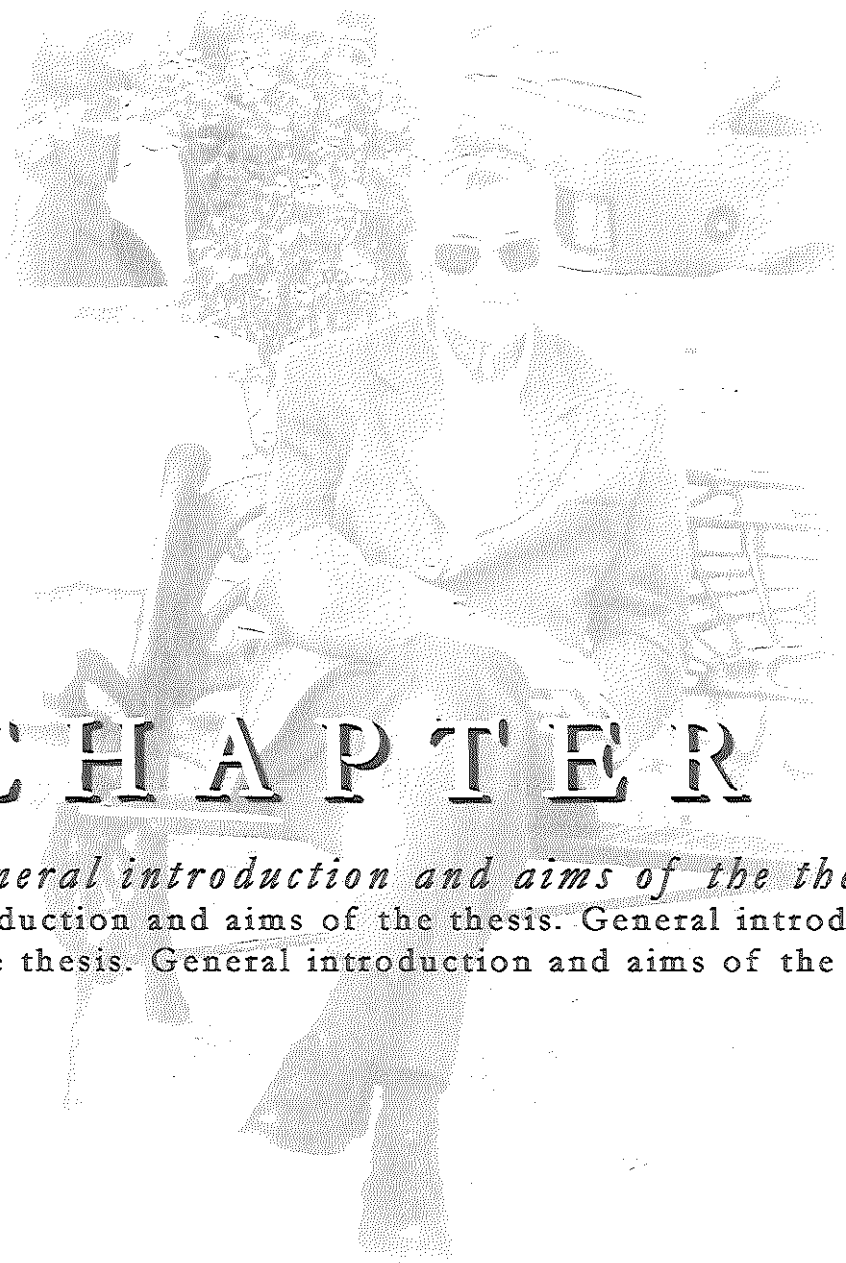
Chapter 8

M. van den Akker, A.W. van den Beld, K.W. Redekop and S.W.J. Lamberts. Relationships between hormone levels, functional status, and health-related quality of life amongst elderly, predominantly self-sufficient Dutch men. Submitted for publication

Chapter 9

A.W. van den Beld, M.L. Bots, R.A. Feelders, D.E. Grobbee, T.J. Visser, W.F. Blum, H.F.J. Savelkoul, R.H.N. van Schaik, H.A.P. Pols, and S.W.J. Lamberts. Hormonal, inflammatory and physical predictors of mortality in a population of healthy elderly men. Submitted for publication.



CHAPTER 1

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The average length of human life is currently 75 to 78 years and may increase to 85 years during the coming two decades (1), but it is not clear whether these additional years will be satisfying to live. Most data indicate modest gain in the number of healthy years lived but a far greater increase in years of compromised physical, mental and social function (2). The number of days of restricted activity and the number of admissions to hospitals and nursing homes sharply increases after age 70 (3). One U.S. health interview survey indicates that, at present, more than 25 million aging people suffer from physical impairment, whereas the number of people requiring assistance in activities of daily living increases from 14% at age 65 to 75 to 45% in those over 85 years old (4).

Aging and Physical Frailty

Throughout adult life, all physiological functions gradually decline (5). There is a diminished capacity for cellular protein synthesis, a decline in immune function, an increase in fat mass, a loss of muscle mass and strength, and a decrease in bone mineral density (5). Most elderly individuals will die from atherosclerosis, cancer or dementia; but in an increasing number of the healthy oldest old, loss of muscle strength is the limiting factor that determines their chances of living an independent life until death.

Age-related disability is characterized by generalized weakness, impaired mobility and balance and poor endurance. In the oldest old, this state is called physical frailty, which is defined as "a state of reduced physiological reserves associated with an increased susceptibility to disability" (6). Clinical correlates of physical frailty include falls, fractures, impairment in activities of daily living, and loss of independence; falls contribute to 40% of admissions to nursing homes (7).

Loss of muscle strength is an important factor in the process of frailty. Muscle weakness can be caused by aging of the muscle fibers and their innervation, osteoarthritis, and chronic debilitating diseases (8). However, a sedentary lifestyle and decreased physical activity and disuse are also important determinants of the decline in muscle strength. In a study of 100 frail nursing home residents (average age 87 years), lower-extremity muscle mass and strength were closely related (9). Supervised resistance exercise training (for 45 min three times per week for 10 weeks) doubled muscle strength and significantly increased gait velocity and stair-climbing power. This demonstrates that frailty in the elderly is not an irreversible effect of aging and disease but can be reduced and perhaps even prevented (9). Also, among non-disabled elderly people living in the community, objective measures of lower-extremity function are highly predictive of subsequent disability (10). Prevention of frailty can be achieved only by exercise. However, exercise is difficult to implement in the daily routine of the aging population, and the number of dropouts from exercise programs is very high (11).

Part of the aging process affecting body composition (loss of muscle size and strength, loss of bone, and increase in fat mass) might also be related to changes in the endocrine system (6,12).

Endocrinology of Aging

One of the clinically most important changes in endocrine activity during aging involves the pancreas. Approximately 40% of individuals 65 to 74 years old and 50% of individuals older than 80 years have impaired glucose tolerance or diabetes mellitus, and nearly half of elderly diabetics are undiagnosed (13,14). These persons are at risk of developing secondary, mainly macrovascular, complications at an accelerated rate (15). Apart from decreased (relative) insulin secretion by the β cells, peripheral insulin resistance related to poor diet, physical inactivity, increased abdominal fat mass, and decreased lean body mass contribute to the deterioration of glucose metabolism (15,16).

The changes in insulin sensitivity that occur in the aging population are frequently of clinical importance and are recognized and treated as diseases. Three other hormonal systems show decreasing circulating hormone concentrations during normal aging, and these changes have been considered mainly physiological (Figure 1). In recent years, hormone replacement strategies have been developed, but many of their aspects remain controversial, and increasing hormone blood levels to those found in 30- to 50-year-old individuals has not yet been uniformly proven to be safe and of benefit.

The most dramatic and rapidly occurring change in women around the age of 50 is menopause (17). Cycling estradiol (E2) production during the reproductive years is replaced by very low, constant E2. For many years, the prevailing view was that menopause resulted from an exhaustion of ovarian follicles. An alternative perspective is that age-related changes in the central nervous system and the hypothalamo-pituitary unit initiate the menopausal transition. The evidence that both the ovary and the brain are key pacemakers in menopause is compelling (18). Changes in the activity of the hypothalamo-pituitary-gonadal axis in males are slower and more subtle. During aging, a gradual decline in serum total and free testosterone (T) levels occurs (19). This "andropause" is characterized by a decrease in testicular Leydig cell numbers and in their secretory capacity, as well as by an age-related decrease in episodic and stimulated gonadotropin secretion (20).

The second hormonal system demonstrating age-related changes is the circulating levels of dehydroepiandrosterone (DHEA) and its sulphate (DHEAS), which gradually decline with age, resulting in "adrenopause" (21,22). Adrenal secretion of DHEA gradually decreases over time, whereas adrenocorticotropin (ACTH) secretion, which is physiologically linked to plasma cortisol levels, remains largely unchanged. The decline in DHEA(S) levels in both sexes contrasts therefore with the maintenance of plasma cortisol levels and seems to be caused by a selective decrease in the number of functional zona reticularis cells in the adrenal cortex rather than regulated by a central (hypothalamic) pacemaker of aging (21).

The third endocrine system that gradually declines in activity during aging is the growth hormone (GH)/insulin-like growth factor I (IGF-I) axis (23). Mean pulse amplitude, duration, and free fraction of GH secreted, but not pulse frequency, gradually decrease during aging. In parallel, there is a progressive fall in circulating IGF-I levels in both sexes (23). There is no evidence for a "peripheral" factor in this process of "somatopause", and its triggering pacemaker seems mainly localized in the

hypothalamus, because pituitary somatotrophic cells, even of the oldest old, can be restored to their youthful secretory capacity during treatment with GH-releasing peptides.

We do not know whether the changes in gonadal function (menopause and andropause) are interrelated with the processes of adrenopause and somatopause, which occur in both sexes. In addition, functional correlates (such as muscle size and function, fat and bone mass, progression of atherosclerosis, and changes in cognitive function) have not been related to these changes in endocrine activity. However, a number of effects of normal aging closely resemble features of (isolated) hormonal deficiency (such as hypogonadism and GH deficiency), which in mid-adult patients are successfully reversed by replacement therapy with the appropriate hormone (24). Although aging does not simply result from a variety of hormone deficiency states, medical intervention in the processes of meno-, andro-, adreno-, or somatopause might successfully prevent or delay some aspects of the aging process.

"Andropause"

Luteinizing hormone (LH) is secreted in pulses by the pituitary after stimulation by LH releasing hormone (LHRH) secreted by the hypothalamus. Pulsatile secretion of follicle stimulating hormone (FSH) is temporally coupled to that of LH but lower in amplitude. LH stimulates testosterone secretion by the Leydig cells of the testis. The control of LH in men operates primarily by negative feedback of both testosterone and estradiol.

Both cross-sectional (19) and longitudinal (25) studies have shown that in healthy males mean serum total testosterone (T) levels decrease by about 30% between age 25 and 75, whereas mean serum free T (FT) levels decrease by as much as 50% over the same period. The variability in circulating (free) T levels among elderly is considerable, however. The steeper decline of FT levels compared to that of total T levels is explained by an age-associated increase in sex-hormone binding globulin (SHBG) binding capacity (26). Genetics appear to play an important role in determination of T levels. The age-related decrease in T secretion in elderly men is the consequence of both primary testicular changes (decreased number of Leydig cells), and an altered neuroendocrine regulation of Leydig cell function with inappropriate LH secretory response to prevailing hypoandrogenism. In the presence of an inadequate neuroendocrine response to decreased T levels, the independent progressive increase of plasma SHBG binding capacity may then cause an even sharper fall of FT levels (26).

Numerous studies of large populations of healthy men have shown a marked rise in the incidence of impotence to over 50% in men aged 60 to 70 (27). Although this increase in impotence seems to occur in the same age group that shows a clear decline in free T levels, no causal relationships have been demonstrated. Testosterone replacement therapy is in most instances not effective for the treatment of impotence in elderly males; other factors such as atherosclerosis, alcohol consumption, smoking, and the quality of personal relationships seem to be more important denominators (28).

and fragility, impaired functioning, and chronic diseases (such as osteoporosis, diabetes, cancer, and heart disease) have not been studied yet.

"Adrenopause"

Dehydroepiandrosterone (DHEA) in its free, sulfated or lipoidal form is the most abundant steroid secreted by the zona reticularis of the adult human adrenal. Circulating DHEAS levels in healthy adults are more than 10 times higher than those of cortisol (22). It is well known that aging is associated with a marked decrease of the adrenal C_{19} steroid DHEA and its sulfate (39,40). At age 30, DHEAS levels are approximately five times higher than at age 85. DHEA is a universal precursor for androgenic and estrogenic steroid formation in peripheral tissues, which contain a number of DHEA-metabolizing enzymes (21). A variety of factors influence cortisol and DHEA levels; obesity, meals, insulin, stress, alcohol and smoking alter adrenal steroid levels.

The knowledge about the functions of adrenal C_{19} steroids is mainly derived from animal studies. Animal studies in rodents, which have very low circulating DHEAS levels, suggest that DHEA administration prevents obesity, diabetes mellitus, cancer and heart disease, while enhancing immune function (21). These results suggest that DHEA prolongs life-span and might be an "elixir of youth". Supportive data in humans are few, highly controversial and unresolved. Higher DHEAS levels are accompanied by a modestly reduced risk of death from cardiovascular disease in males (41). Functional parameters of daily living in males over 90 years old were lowest in those with the lowest DHEAS levels (22).

Two randomized placebo-controlled studies support the concept that oral administration of DHEA has beneficial effects (42,43). Three months of daily treatment with 50 mg of DHEA of 20 adults, most of whom were non-elderly individuals (40 to 70 years old), increased DHEA(S) levels to young adult levels, increased plasma androgen and IGF-I concentrations, and induced a remarkable increase in perceived physical and psychological well-being in both sexes without an effect on libido. However, several other studies on DHEA replacement in humans, yielded inconsistent and controversial results (44-47). DHEA might influence the central nervous system activity (48). However, whether DHEA is related to cognitive function remains controversial.

It is unknown whether the increase in sex steroid levels induced by DHEA is safe with regard to the development of prostate or other types of cancer. DHEA is currently widely used in the United States as a "treatment for aging". With the scientific verdict still out, without confirmation of DHEA's reported beneficial actions in humans, and without a better understanding of its potential risks, it is premature to recommend the routine use of DHEA for delaying or preventing the physiologic consequences of aging (49).

"Somatopause"

Growth hormone (GH) is a single chain polypeptide, which is produced by the pituitary gland. It is an important anabolic hormone with stimulatory effects on protein

synthesis and on lipolysis. GH secretion is regulated by the interaction of two hypothalamic peptides, GH-releasing hormone (GHRH) and somatostatin. GH has both direct and indirect actions on peripheral tissues. Indirect effects of GH are mediated mainly by insulin-like growth factor (IGF) I, generated in the liver. IGF-I, in turn, has a negative feedback action on the hypothalamus and pituitary to inhibit GH release. IGF-I as well as IGF-II play a pivotal role in the regulation of proliferation, differentiation and specific functions of many cell types. The major regulators of IGF-I are growth hormone (GH) and nutrition, although its production in specific tissues is regulated by a multitude of tropic hormones and other peptide growth factors. IGFs are bound to specific binding proteins (IGFBP). The six IGFBPs which are known at present either bind IGF-I and IGF-II with similar affinities or show a preference for IGF-II. Each of the IGFBPs has a unique role as a modulator of IGF action. The predominant IGFBP in the blood is IGFBP-3 which is part of a large molecular weight ternary complex. IGFBP-3 is mainly regulated by GH. Circulating IGFBP-3 levels reach a maximum at puberty and decrease between 18 and 79 years in both sexes (23). It increases the half-life of IGF-I by a 100 fold and acts as an inhibitor as well as a stimulator of IGF-I action. In addition, the acid-stable IGFBP-3 subunit may have mitogenic activity independent of IGF-I itself (50). The secondary most abundant IGFBP in the circulation is IGFBP-2. Its affinity for IGF-II is an order of magnitude higher than its affinity for IGF-I. IGFBP-2 is regulated by several mechanisms. It decreases after birth until puberty, after which it gradually increases again, especially after the age of 60 (51). At age 80, concentrations are nearly twice as high as in young adults. Serum IGFBP-2 concentrations are higher during starvation, fasting and protein restriction, as well as in growth hormone deficiency. IGFBP-2 has been shown to inhibit IGF-I receptor binding and action (52), although its cellular action in IGF-mediated functions also has reported to be stimulatory. IGFBP-1 is GH-independent, increases with fasting and is most prominently characterized by its inverse relationship to plasma insulin concentrations. IGFBP-1 is most strongly expressed in liver and kidney. IGFBP-1 is thought to modulate the free fraction of circulating IGF, in part by its stimulating transcapillary transport of IGFs to the extravascular space, a process promoted by insulin (53).

In man both aging and GH deficiency are associated with reduced protein synthesis, decreases in lean body mass and bone mass, and increases in body fat (23). The reduction of circulating GH levels during aging (54,55) is due mainly to a reduction in pulse amplitude (56). Plasma IGF-I concentrations are reduced in healthy older adults, although there is wide individual variation. The IGF-I reduction probably results from a reduced stimulus of the liver to produce IGF-I rather than an age-related insensitivity or inability of the liver to respond to circulating GH.

Little is known concerning the biological consequences of somatopause (57). In several studies of healthy individuals of a broad age range, an association was observed between the maximum aerobic capacity and circulating IGF-I levels (58). The expectation that somatopause contributes to the decline of functional capacity in the elderly is mainly derived from studies in which GH replacement therapy of GH-deficient adults was shown to increase muscle mass, muscle strength, bone mass, and quality of life. A

beneficial effect on the lipid profile and an important decrease in fat mass were also observed in such patients (59). The GH/IGF-I axis may also play a role in the age-related decline of certain cognitive functions (60). As in hypogonadal individuals, GH deficiency in adults is considered a model of normal aging, because a number of catabolic processes that are central in the biology of aging can be reversed by GH administration.

GH administration for 3 to 6 months to healthy elderly individuals increased IGF-I levels to those observed in control individuals half their age, while muscle mass, skin thickness, and bone mineral content significantly increased and fat mass decreased (61,62). A disappointing aspect of the studies is that no positive effect of GH therapy was observed on muscle strength (63), while controversial results are reported on the effect on maximal oxygen consumption (63,64). If GH was administered in combination with resistance exercise training, however, a significant positive effect on muscle mass and muscle strength was recorded that did not differ from that seen with placebo treatment, which suggests that GH does not add to the beneficial effects of exercise (65,66).

Earlier studies have demonstrated that pharmacological doses of GH prevent the "auto-cannibalistic" effects of acute diseases on muscle mass (67). This prompted us to carry out a randomized placebo-controlled trial of 6 weeks of GH administration in elderly individuals wit an acute hip fracture. Our results indicate that in individuals over 75 years old, GH administration causes a statistically significant earlier return to independent living after the fracture (68). Comparable studies are currently being done in several countries, and confirmation is needed before GH might gain a place in the treatment of acute catabolic states in the frail elderly.

Other components in the regulation of the GH/IGF-I axis are effective in activating GH and IGF-I secretion. Long-acting derivates of the hypothalamic peptide growth hormone releasing hormone (GHRH), given twice daily subcutaneously for 14 days to healthy 70 years old men, increased GH and IGF-I levels to those encountered in 35-years-olds (54). These studies support the concept that somatopause is primarily hypothalamically driven and that pituitary somatotrophic cells retain their capacity to synthesize and secrete high levels of GH. GH releasing peptides (GHRPs) are oligopeptides with even more powerful GH-releasing effects (69). Originally developed by design, it has recently been suggested that they mediate their GH-secretory effects through endogenous specific receptors (70). Non-peptides analogs such as MK-677 and L-692,429 have powerful GH-releasing effects, restoring IGF-I secretion in the elderly to levels encountered in young adults (71,72). Long-term oral administration of MK-677 to healthy elderly individuals increased lean body mass but not muscle strength. If proven to be GH-specific, these orally active GHRP derivates might be important alternatives to subcutaneously administered GH in the reversal of somatopause, in the prevention of frailty, and in the reversal of acute catabolism. The long-term safety of the activation of GH/IGF-I levels remain uncertain with regard to tumor growth, as most human solid cancers express IGF-I receptors (73).

Thyroid function

Age-related thyroid dysfunction is also common in the elderly (74). Lowered plasma thyroxine (T4) and increased thyrotropin-stimulating hormone (TSH) concentrations occur in 5 to 10% of elderly women (74). These abnormalities seem to be mainly caused by autoimmunity and may therefore be an expression of age-associated disease rather than a consequence of the aging process (75,76). Normal aging is accompanied by a slight decrease in pituitary TSH release (74), but especially by a decreased peripheral degradation of T4, which results in a gradual age-dependent decline in serum triiodothyronine (T3) concentration, without an important change in T4 levels (77). This slight decrease in plasma T3 concentration occurs largely within the broad normal range of the healthy elderly population and has not been convincingly related to functional changes during the aging process (74).

The production of thyroxine (T4) and triiodothyronine (T3) by the thyroid is stimulated by thyroid-stimulating hormone (TSH), which is secreted by the pituitary. TSH release, in turn, increases in response to thyrotropin-releasing hormone (TRH), produced by the hypothalamus. T4 and T3 inhibit both TSH and TRH secretion by a negative feedback mechanism. T4 is considered as a pro-hormone of T3, which is the metabolically active hormone. Thyroidal T3 release accounts for 20% of daily T3 production, whereas 80% is formed via extra-thyroidal deiodonation of T4. Peripheral conversion of T4 to T3 occurs predominantly in the liver and is mediated through deiodination of the outer ring of T4, catalyzed by an enzyme termed type I deiodonase. Inner ring deiodination converts T4 to reverse triodothyronine (rT3), an inactive metabolite. Inner ring or outer ring deiodination of T3 and rT3 respectively result in the formation of diiodothyronine (T2). In the circulation, thyroid hormones are bound to plasma proteins, in particular thyroxine-binding globulin (TBG) and, to a lesser extent, albumin and pre-albumin. The biological actions of thyroid hormones are thought to be mediated by the free fractions of T4 and T3 (78). Thyroxine sulfate (T4S) is another inactive metabolite of T4. T4S is produced mainly in peripheral tissues equipped with appropriate sulfotransferases, e.g. liver, brain, platelets, and granulocytes (79-81). T4S levels are not changed significantly in hyperthyroidism or hypothyroidism.

It is well recognized that symptoms of aging can be easily confused with hypothyroidism, and in the past decreased thyroid function was believed to be one of the hall-marks of the aging process (82). During aging a complex number of changes occur in thyroid hormone concentrations. Reduced outer ring deiodination mediated by type I deiodinase results in a decline in T4 degradation, with a reduced generation of T3 and a decreased clearance of rT3. In addition TSH secretion appears to be slightly decreased in healthy elderly humans when subjects with subclinical hypothyroidism are carefully excluded. The reason for such age-dependent reduction of TSH is uncertain (74). However, in spite of these complex changes in biochemical parameters, recent studies suggest that normal aging is associated with an essentially normal thyroid function, although evaluation of thyroid function in the elderly is confounded by the increased prevalence of autoimmune subclinical hypothyroidism and non-thyroidal illness (83).

Inflammation

Frailty is contributed to by, at least, chronic and acute diseases, the physiological decline that occurs during the aging process, and a dysregulation of systems that also appears to accompany aging (84). Also the immune system seems to play a role in frailty. The role of inflammation in the pathogenesis of atherothrombosis (85) and other acute and chronic conditions has been well described (86,87). However, recently it has been observed that markers of inflammation, such as C-reactive protein (CRP) and interleukin-6 (IL-6) levels may have broader applicability as indicators of disability and mortality (88,89). Aging is associated with an elevation in pro-inflammatory cytokines, such as IL-6 (90), which play a central role in the hepatic production of CRP and other acute-phase proteins involved in the inflammatory response (91). It is hypothesized that in older adults a chronic inflammatory state may contribute to the pathophysiology of medical conditions that result in functional decline and disability.

The Concept of Successful Aging

There is considerable variation in the effects of aging in healthy individuals, with some persons exhibiting extensive alteration in physiological functions with age and others little or none. It has been suggested that it might be useful to distinguish between usual and successful patterns of aging (92). Genetic factors, lifestyle, and societal investments in a safe and healthful environment are important aspects of successful aging (93). Traditionally, the aging process, including the development of frailty toward the end of life, has been considered to be physiological and unavoidable. In recent years, however, it has become evident that it might not be necessary to accept the grim stereotype of aging as an unalterable process of decline and loss (9,92). The concept frailty focuses mainly on the physical or physiological aspects of aging, while the concept of successful aging comprises a broader range of aspects. Both concepts are not easy to define in a single measure. As life expectancy will increase further in coming decades (94), the overarching goal for the year 2000 and thereafter should be "an increase in years of healthy life with a full range of functional capacity at each stage of life". Such a compression of morbidity can often be achieved through lifestyle measures, but a number of aspects of the aging process of the endocrine system invite the development of "routine" medical intervention programs offering long-term replacement therapy with one or more hormones, in order to delay the aging process and to allow us to live for a longer period in a relatively intact state.

Scope of the thesis

In this thesis the definition of successful aging as illustrated in the model by Rowe, with 'maintaining high cognitive and physical functional capacity', 'avoiding disease and disability', and 'maintaining active engagement with life' as main determinants (Figure 2), will be taken as a starting point in order to obtain more insight in the value and the interactions of the different aspects which are important to age successfully. For an individual assessment of successful aging adequate, reproducible, and if possible, predictive measures are needed. Therefore one of the aims of our study is to find

parameters which reliably reflect the three different parts of the model. To measure overall successful aging we will use two parameters; quality of life and survival.

Since part of the aging process seems to be related to the endocrine system and because markers of inflammation may have an applicability as indicators of disability and mortality, another aim of this study is to investigate the role of the hormonal system and inflammation in different parts of successful aging.

In principle successful aging should be investigated in both sexes. However, logistically it is complex to investigate it in both sexes, since it doubles the number of subjects which should be examined. In addition, there will be slightly different questions in men compared to women with regard to successful aging. Therefore we only focused on men in this study.

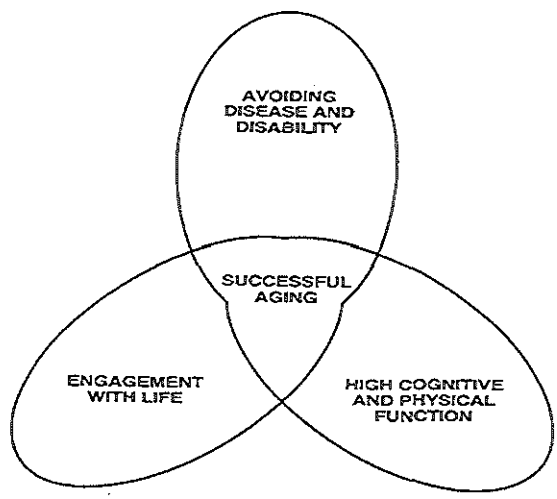

Figure 2. The concept of Successful Aging as illustrated by Rowe with 'maintaining high cognitive and physical functional capacity', 'avoiding disease and disability', and 'maintaining active engagement with life' as main determinants.

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CHAPTER 2

ly men. *Serum insulin-like growth factor binding* and pro
r binding *protein-2 levels as an indicator of* functional abi
cator of *functional ability in elderly men.* Serum insulin-li

ABSTRACT

Background In a cross-sectional study in 403 healthy, independently living elderly men (mean age 78 years), we determined which are the main physiological determinants of functional ability in the elderly, and which components of the somatotropic system contribute to the maintenance of functional ability.

Methods Functional ability was assessed by the number of problems in activities of daily living and by a measure of physical performance. Other physical characteristics included leg extensor strength, bone mineral density of total body and proximal femur and body composition, including lean mass and fat mass. Serum insulin-like growth factor (IGF)-I and its binding proteins (IGFBP) -1, -2 and -3 concentrations were all measured by RIA.

Results Muscle strength was positively related to activities of daily living, which indicates a lower degree of disability. It was also positively related to physical performance and bone mineral density (all $P<0.001$). Fat mass influenced activities of daily living and physical performance negatively and bone mineral density positively (all $P<0.001$). Serum concentrations of IGF-I and IGFBP-3 were not related to any of the physical characteristics. Serum IGFBP-2 concentrations were negatively related to activities in daily living ($P<0.001$), physical performance ($P=0.006$), muscle strength ($P=0.002$), bone mineral density of proximal femur ($P=0.007$), lean mass and fat mass (both $P<0.001$). Serum insulin concentrations were positively and IGFBP-1 concentrations negatively related to lean mass ($P<0.001$) and fat mass ($P<0.001$).

Conclusions In independently living elderly men, functional ability appears to be determined by muscle strength (positive) and fat mass (negative). Low serum IGFBP-2 concentrations are a powerful indicator for an overall good physical functional status, probably inversely reflecting the integrated sum of nutrition and the biological effects of growth hormone, IGF-I and insulin.

INTRODUCTION

Traditionally, the aging process has been considered to be physiological and unavoidable. Loss of muscle mass and strength are important predictors of a decrease in physical performance (1). Physical frailty, which is defined as "a state of reduced physiologic reserves associated with increased susceptibility to disability" (2), frequently occurs towards the end of life, and often results in the loss of independence. In recent years it has become evident that it might not be necessary to accept the grim stereotype of aging as an unalterable process of decline and loss (1,2). Since many of the predictors of physical functional status appear to be potentially modifiable, research must be done to refine diagnostic criteria and develop practical methods of measurement of key physiological capacities in order to identify proper targets for interventions in disabled elderly or for preventive interventions in 'normal' elderly individuals, with the ultimate goal to enhance the proportion of the older population that ages successfully. Prevention of loss of physical functions can be achieved by exercise (1), while hormone replacement with growth hormone (3-6) in the elderly has been demonstrated to improve physical performance in selected groups of elderly individuals. Recently, also data have been reported which suggest that the IGF / IGF-binding protein system might be related to the presence or development of prostate malignancies (7).

With regard to the study of the age-related decline of physical function and its relationships with the endocrine system, we investigated two central questions in the present study: Which are the main physiological determinants of functional ability in the elderly and: Which components of the somatotropic system contribute to the maintenance of functional ability. Also a possible relationship between prostate disorders and the serum concentrations of these hormones was studied. We have investigated these questions in a cross-sectional study in 403 healthy, independently living elderly men.

METHODS

Subjects.

A cross-sectional, single-center study was conducted in 403 independently living men, aged 70 years and higher. Names and addresses of all male inhabitants 70 years and older were drawn from the municipal register of Zoetermeer, a medium sized town in the mid-western part of the Netherlands. 1567 men were invited. A total of 886 men did not respond to the mailed invitation in which it was mentioned that only subjects who lived independently and had no severe mobility problems could participate. After exclusion of subjects who did not live independently and subjects who were not physically or mentally able to visit the study center independently, eventually 403 men participated (25.7%). The main reason not to participate among the respondents was because they were currently under the care of a medical specialist or general practitioner (28%), while 16% was excluded on the basis of physical (10%) or mental (6%) problems. Participants signed an informed consent. The study has been approved by the Medical Ethics Committee of the Erasmus University Hospital Rotterdam. No additional health related eligibility criteria were used. A

number of participants were taking medications for chronic illnesses, including hypertension (n=96) and mild congestive heart failure (n=28). However, in retrospect, none of these medications did influence the relations described in this study. Some of the illnesses, for example mild knee pain (n=79), influenced the physical characteristics measured, but they did not change the relations between the physical characteristics, nor between the circulating hormone levels and the physical characteristics reported in this study.

Hormone measurements

Blood samples were collected in the morning after an overnight fast. Serum was separated by centrifugation and deeply frozen. The period of storage at -40°C varied from 0 till 5 months. Total insulin-like growth factor-1 was measured by an insulin-like growth factor binding protein (IGFBP) blocked radioimmunoassay (RIA) as described previously (8). IGFBP-1, IGFBP-2 and IGFBP-3 were determined by RIA as described previously (9-11). Insulin was measured by a commercially available RIA (Pharmacia, Freyburg, Germany).

Physical characteristics

Physical Performance

Lower extremity function, or physical performance, was assessed as described by Guralnik et al. (12), including measurements of standing balance, walking speed and ability to rise from a chair. The three tests of standing balance were considered in hierarchical difficulty by assigning a single score of 0 to 4 for standing balance. For the 8-foot walk and repeated chair stands, those who could not complete the task were assigned a score of 0. Those completing the task were assigned scores of 1 to 4, corresponding to the quartiles of time needed to complete the task, with the fastest times scored as 4. A summary performance scale was created by summing the category scores for the walking, chair stand, and balance test, which ranged from 0 (worst performance) to 12 (best performance). Mean scores of the three tests as well as of the summary performance scale measured in this study were comparable to those reported in subjects of the same age group investigated by Guralnik (12).

Activities of daily living

Self-reported disability or satisfaction in performing activities of daily living was assessed by a self-administered questionnaire modified from the Stanford Health Assessment Questionnaire as described by Pincus et al (13). A score of 8 points was obtained if the participants reported no problems in activities of daily living, needed no help and when there was no difference compared with the situation 6 months ago. A minimum score of 0 was given if participants improved compared to 6 months previously, while a maximum score of 36 points was assigned to participants who reported severe problems in activities of daily living, needed help and had more problems in activities of daily living compared to 6 months ago. The lower the points measured in this ADL score, the lower the degree of disability.

The Mini-Mental State Examination was used to determine cognitive function (14).

Muscle Strength

Isometric leg extensor strength was measured as described by Hsieh and Philips using the Hoggan MicroFET hand held dynamometer (15). The measurement requires that the participant, in a seated position, pushes with maximal strength to the dynamometer, which is held at the tibia preferably 33 cm below the knee joint. The investigator holds the dynamometer in the hand with an extended arm and pushes back until the breaking point is reached. The measurements were done in two positions and at both legs. During the first series of measurements the leg was held in 120° extension and during the second series of measurements the leg was in extended position (180°). During the first series of measurements the participant holds a device over the leg tested, which keeps the leg in exactly 120°. The measurements were done three times and the maximum performance at each position was recorded. To obtain one measure of leg muscle strength, "maximum leg extensor strength" was defined as the maximum strength for the right or left leg in a position of 120°. Statistical analyses were based on the physical unit measurement moments (N*m), obtained by multiplying the maximum strength (in Newton) and the distance of the dynamometer to the knee joint (in meters).

Bone mineral density and Body Composition

Total body bone mineral density was measured using dual energy x-ray absorptiometry (DXA, Lunar, Madison, WI), as were hip bone mineral densities at the femoral neck, trochanter and Ward's triangle. In addition total lean body mass and fat mass were measured (16,17). Quality control including calibration was performed routinely every morning for DXA, using the standard provided by the manufacturer.

Height and weight were measured in standing position without shoes. Body mass index was calculated as the weight in kilograms divided by the square of the height in meters.

Measurements of prostate

Medical history on signs of enlarged prostate were obtained. Rectal examination was performed. Prostate Specific Antigen (PSA) was determined by a commercially available kit.

Data analyses

Results are expressed, unless otherwise stated, as mean and standard deviation with the interquartile (IQ) range. Variables which were not normally distributed were logarithmically transformed. Relations between variables were assessed using linear regression for continuous variables, stated as linear regression coefficient (β) and standard errors. Multiple regression analysis was used to adjust for age and body mass index, as well as to assess the contribution of different independent variables to the dependent variable. Partial correlations between variables were assessed by calculating Pearson's product r. Unless otherwise mentioned all analyses are done after adjustment for age. Analyses were performed using Stata statistical package (StataCorp. 1997. Stata Statistical Software: Release 5.0).

RESULTS

Characteristics of the study population are given in Table 1. Mean age of the study population was 77.8 yr (Standard Deviation 3.58).

Relations between age and the different physical characteristics

Physical performance, muscle strength, bone mineral density, lean body mass and fat mass all decreased with age (Table 2). With increasing age, more limitations in activities of daily living were reported.

Table 1. Descriptive Data of the study population

	Means	SD	IQ-range	
Age (yr)	77.8	3.6	75	80
Body Mass Index	25.5	3.0	23.3	27.3
Physical Performance (points)	8.5	2.4	7	10
Activities of daily living (points)	10.7	4.3	8	12
Total Body Bone Mineral Density (g/cm^2)	1.17	0.10	1.11	1.23
Femoral Neck Bone Mineral Density (g/cm^2)	0.88	0.14	0.78	0.97
Femoral Ward Bone Mineral Density (g/cm^2)	0.72	0.16	0.60	0.82
Femoral Trochanter Bone Mineral Density (g/cm^2)	0.85	0.15	0.76	0.94
Total Fat Mass (kg)	21.2	6.4	17.4	24.5
Total Lean Mass (kg)	51.7	5.6	47.8	55.5
Maximum Leg Extensor Strength (Nm)	103.2	20.9	89.4	117.

Table 2. Relations between physical characteristics and age

	Age		
	β	± S.E.	P
Physical Performance Score (points/yr)	-0.22	0.03	<0.001
Activities in Daily Living (log)	0.01	0.005	0.02
Total Body Bone Mineral Density ($g/cm^2/yr$)	-0.01	0.001	<0.001
Total Fat Mass (kg/yr)	-0.18	0.09	0.05
Total Lean Mass (kg/yr)	-0.38	0.08	<0.001
Maximum Leg Extensor Strength (Nm/yr)	-1.54	0.28	<0.001

β regression coefficient denotes changes in unit per year. For example: Physical Performance score decreases 0.22 point per year.

Relationships among the different physical characteristics

Relations among the different physical characteristics, estimated by linear regression analysis, are illustrated in Figure 1. Subjects that scored better in the physical performance test had significantly less problems in activities of daily living (i.e. a lower disability) and significantly higher bone mineral density (at all sites measured). Muscle strength was positively, independently related to the physical performance score and bone mineral density at all sites measured, while muscle strength and the number of problems in activities of daily living were inversely related. This implies that subjects with high muscle strength reported significantly less problems with activities of daily living (lower disability), scored significantly better in the physical performance test and had significantly higher bone mineral density compared to subjects with low muscle strength. Further, lean body mass was strongly associated with muscle strength.

Fat mass was inversely related to the physical performance score, but independently positive with problems in activities of daily living and total body as well as proximal femur bone mineral density. This implies that subjects with a high fat mass scored significantly lower on the physical performance test, had more problems with activities of daily living, but had significantly higher bone mineral density compared to subjects with low fat mass.

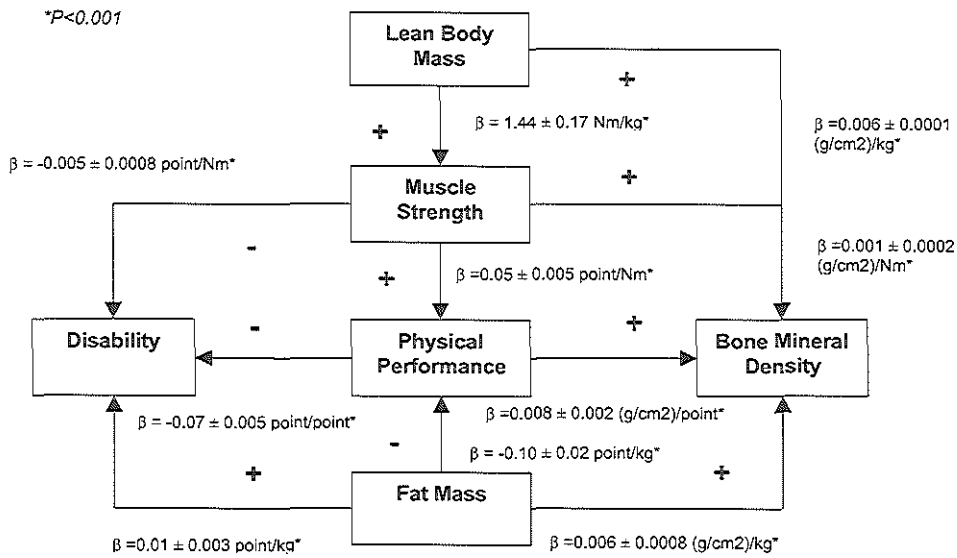
Relations between the hormones and age

Summarized values of the hormones are presented in Table 3. Serum concentrations of Insulin-like Growth Factor -I (IGF-I) and its binding protein-3 (IGFBP-3) decreased with age (β=-0.85 ± 0.41 (μg/l)/year, P=0.04, and β=-0.07 ± 0.01 (mg/l)/year, P<0.001, respectively). Serum concentrations of IGFBP-1 and IGFBP-2 increased with age (β=0.03 ± 0.006 (μg/l)/year, P<0.001 and β=0.26 ± 0.04 (mg/l)/year, P<0.001, respectively). Serum insulin levels were not associated with age.

Table 3: Summarized values of serum hormone concentrations

	mean	S.D.	IQ-range
IGF-I (μg/l)	100.9	29.2	81.2 - 118.9
IGFBP-1 (μg/l)	31.7	15.5	21.2 - 38.6
IGFBP-2 (mg/l)	0.62	0.32	0.40 - 0.76
IGFBP-3(mg/l)	2.59	0.70	2.09 - 3.04
Insulin (IU/l)	8.91	4.25	6.07 - 10.5

Figure 1. Relations between the physical characteristics. β denotes linear regression coefficient. F.e. Physical performance increases 0.05 ± 0.01 point per Nm muscle strength. * denotes P-value <0.001. Since physical performance, activities in daily living, bone mineral density and muscle strength were all associated with body mass index, analysis including these parameters were done after adjustment for body mass index (and age). Activities in daily living were not normally distributed; therefore activities of daily living were included in the analyses after logarithmic transformation. The linear regression coefficient of the relation between muscle strength and activities of daily living is negative: the lowest scores in activities in daily living (i.e. few problems) were reported in men with the highest muscle strength. Thus, a higher muscle strength is associated with a better performance in activities of daily living and therefore with less disability. Part of the relation between physical performance and bone mineral density was explained through muscle strength.



Relationships amongst serum somatotropic hormone concentrations

Serum IGF-I and IGFBP-3 concentrations were strongly and positively related (r=0.53, P<0.001). Serum IGF-I concentrations were inversely related to those of IGFBP-1 (r=-0.17, P<0.001) and IGFBP-2 (r=-0.19, P<0.001). However, as the IGFBP-1 and IGFBP-2 concentrations were interrelated (r=0.49, P<0.001), a multiple regression analysis including IGFBP-1, as well as IGFBP-2, was performed. In this analysis serum IGF-I levels remained only related to those of IGFBP-2. The relations between the serum IGFBP-3 and those of IGFBP-1 and IGFBP-2, respectively, were dependent on IGF-I. Insulin concentrations were independently related to those of IGFBP-1 and IGFBP-2 (r=-0.30 and r=-0.28, P<0.001, respectively).

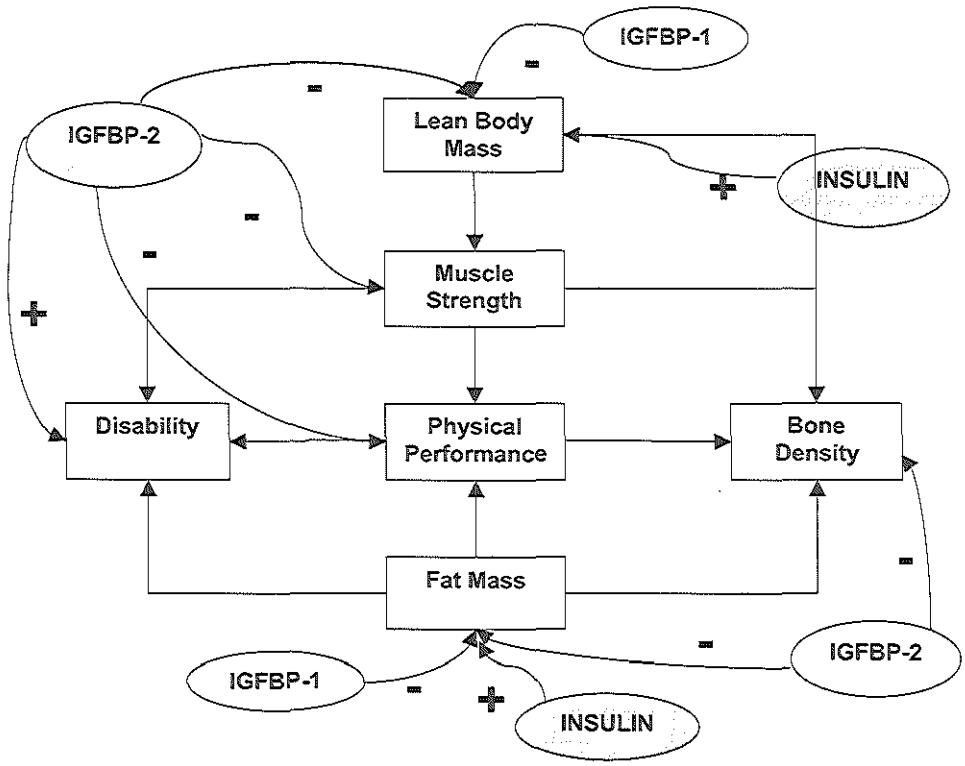
Serum IGF-I and the binding proteins in relation with physical characteristics

Serum IGF-I and IGFBP-3 concentrations were not directly related to any of the physical characteristics studied. In contrast, serum IGFBP-2 levels were significantly, inversely related to virtually all these variables measured, including femur bone mineral density, except for total body bone mineral density (Figure 2). As IGFBP-2 levels were strongly related to body mass index (β= -4.00 ± 0.45 (kg/m^2)/(mg/l), P<0.001), all analyses were repeated after adjustment for body mass index (except for lean and fat mass). Physical performance scores decreased 1.13 ± 0.41 points per mg/l increase in IGFBP-2 (P=0.005). Significantly more problems in activities of daily living were reported with increasing serum IGFBP-2 concentrations (logarithmically transformed: β=0.19 ± 0.06 points/(mg/l), P<0.001). Further, muscle strength decreased 11.33 ± 3.51 Nm per mg/l increase in IGFBP-2 (P=0.001). Bone mineral density of the ward region decreased 0.08 ± 0.03 g/cm^2 per mg/l increase in IGFBP-2 (P=0.005). In addition, both lean body mass and fat mass were lower in individuals with higher IGFBP-2 concentrations (respectively β=-4.09 ± 0.88 kg/(mg/l), and β=-5.58 ± 0.90 kg/(mg/l), both P<0.001). The inverse relation between IGFBP-2 and physical performance was explained through the inverse relation between IGFBP-2 and muscle strength. All the other relations were independent from each other.

Serum IGFBP-1 levels were inversely related to physical performance scores, fat mass and lean body mass, and positively to problems in activities of daily living. However, serum IGFBP-1 and –2 levels were positively related and the relations between IGFBP-1 and physical performance and activities in daily living, respectively, were no longer significant after adjustment for IGFBP-2. The relations between IGFBP-1 and fat mass and lean body mass, respectively, were independent of IGFBP-2 (β=-0.14 ± 0.02 kg/(µg/l) and β=-0.09 ± 0.02 kg/(µg/l), P<0.001 respectively).

Serum insulin concentrations were positively, and independent of serum IGFBP-1 and IGFBP-2 concentrations, related to lean body mass as well as fat mass (β=0.23 ± 0.06 kg/(IU/l) and β=0.40 ± 0.06 kg/(IU/l), P<0.001 respectively).

Figure 2. Relations of serum concentrations of Insulin-like Growth Factor Binding Protein-2, Insulin-like Growth Factor Binding Protein-1, and insulin with the characteristics of physical function. The effects of all hormones mentioned in this figure were independent from each other



Relations between measurements of the IGF-system and prostate

Subjects with a medical history of an enlarged prostate (n=120) or with an enlarged prostate at palpation (n=95) did not have circulating IGF-I, IGFBP-1, -2 and -3 concentrations which differed from the other subjects. In addition, subjects with a suspicion of prostate malignancy at palpation (n=19) or a PSA level above 10 ng/ml (n=34) did not have different IGF-I, IGFBP-1, -2 and -3 concentrations compared to the other subjects. Serum PSA concentrations were also not related with IGF-I, IGFBP-1, -2 or -3 in a linear regression analysis. The proportion of subjects with clinical symptoms of and enlarged prostate, an enlarged prostate at palpation or PSA levels above 10 ng/ml was not different in quartiles of IGF-I, IGFBP-1, -2 and -3. The 35 subjects with a malignancy in the medical history, did not have serum IGFBP-2 concentrations which differed from those of the other subjects.

DISCUSSION

To appreciate the findings of this study, some aspects need to be discussed. In evaluating the physical functional status of the elderly, the choice of the best approach to measure this status remains uncertain. The main clinical correlate used in this study is functional ability, self-reported as well as objectively assessed as a physical performance score using the approach proposed by Guralnik et al. (12). Guralnik demonstrated that a short battery of performance measures of lower extremity function predicts mortality and loss of independence amongst older persons. In addition, bone mineral density, muscle strength and body composition were measured as physical characteristics. Overall physical function, as well as cognitive function in this group of elderly men were relatively good. Only 30 individuals scored less than 24 points in the mini-mental state examination, which indicates mild cognitive impairment. In less selected populations with a broader range of functional ability, if anything, associations are likely to be stronger.

In the present study two major questions in the study of the age-related decline of physical function were addressed. With regard to the first question asked, our findings in this large population of independently living elderly men suggest that high muscle strength and low fat mass are independent determinants of functional ability. This finding agrees with other studies, which demonstrated that muscle strength is independently, positively related to physical performance (1,18) and to bone mineral density (19,20), and inversely to the number of problems in activities of daily living. Fat mass, on the other hand is inversely related to physical performance and restricted activities in daily living, but positively to bone mineral density. This latter relation probably reflects the effect of hormones, such as estradiol or insulin, or simply of mechanical loading. These observations, as well as the other significant relations found in this study, led us to a diagram representing the relationships between activities in daily living, physical performance, bone mineral density, muscle strength and body composition, as summarized in Figure 1.

Part of the age-related physiological changes might be influenced by the activity of the endocrine system (21,22). In this population, physical function declines with age. In parallel, serum IGF-I and IGFBP-3 concentrations significantly decreased with age, while those of IGFBP-1 and IGFBP-2 increased. With regard to the second question, e.g. which components of the somatotropic system contribute to the maintenance of functional ability, we found no correlation between serum IGF-I or IGFBP-3 concentrations on the one hand and measures of functional ability, muscle strength, bone mass and body composition on the other. These findings agree with previous reports (23-28). We did, however, demonstrate highly significant inverse relations between serum IGFBP-2 concentrations and virtually all the physical characteristics measured. IGFBP-2 is present in the human serum in very high concentrations. In adult life IGFBP-2 probably acts to inhibit the biological effects of IGF-I (29). IGFBP-2 is regulated by several mechanisms. It decreases after birth until puberty, after which it gradually increases again, especially after the age of 60 (10,30). At age 80, concentrations are nearly twice as high as in young adults (0.60 mg/l versus 0.35 mg/l). Therefore, the decline in functional ability and the increase in serum IGFBP-2 concentrations proceed in parallel. The precise mechanisms regulating IGFBP-2

concentrations remain unclear, although it is recognized that serum IGFBP-2 concentrations are higher during starvation, fasting and protein restriction, as well as in growth hormone deficiency (31-33), while they respond with a decrease during growth hormone treatment (34,35). In this study, serum IGFBP-2 levels were strongly inversely related to serum albumin concentrations (data not shown), which is generally regarded as a measure of nutritional status (36). In addition, IGF-I appears to be a regulator of IGFBP-2, since IGF-I administration increases its serum levels (37), possibly, however, via a suppression of growth hormone secretion (38). Finally, insulin has been proposed as a regulator of IGFBP-2, although evidence is not yet clear-cut (39). In this study, serum IGF-I, IGFBP-3 as well as fasting insulin concentrations were all inversely related to IGFBP-2 as well. Insulin, which is considered to be an anabolic hormone, was independently of IGFBP-2 related to fat mass and lean mass only. Low serum IGFBP-2 concentrations seem to be related to an anabolic state and turn out to be a powerful indicator of an overall good physical functional status of elderly men. We suggest that serum IGFBP-2 concentrations inversely reflect the integrated sum of the nutritional state, as well as the biological effects of growth hormone, IGF-I as well as insulin.

As IGFBP-2 concentrations have been reported to be elevated in patients with several malignant tumors (40,41), in particular prostate carcinoma (37), one might hypothesize that the association between IGFBP-2 and functional ability is dependent on the presence of prostate malignancies. However, even within the group of individuals with elevated PSA levels, there was no relation between IGFBP-2 and PSA, or other prostate abnormalities. Additionally, in agreement with Ho et al., we did not find abnormal IGFBP-2 concentrations in subjects with an enlarged prostate (42). Consequently, from our study, we do not have indications that IGFBP-2 is produced by the prostate. This implies that the relations between IGFBP-2 and the physical characteristics are not dependent on the presence of prostate hypertrophy or malignancy.

In conclusion, muscle strength and functional ability are considered to be the key characteristics of physical functional status in independently living elderly men. Functional ability appears to be determined by muscle strength (positive) and fat mass (negative). The more marked the functional ability, the higher bone mineral density. Low serum IGFBP-2 concentrations are a powerful indicator for an overall good physical functional status, probably inversely reflecting the integrated sum of nutritional status as well as of the biological effects of growth hormone, IGF-I, and insulin.

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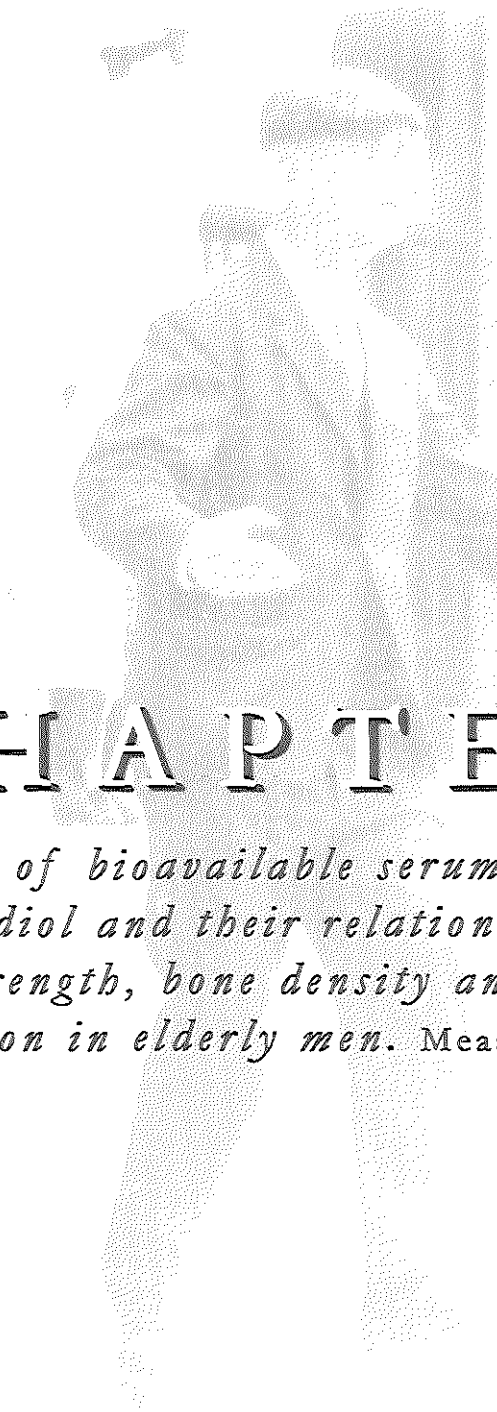

CHAPTER 3

en. *Measures of bioavailable serum testosterone* and estr
ne *and estradiol and their relationships with* muscle stre
th *muscle strength, bone density and body* composition in
dy *composition in elderly men.* Measures of bioavailable ser

ABSTRACT

Background In the present cross-sectional study of 403 independently living elderly men, we tested the hypothesis that the decreases in bone mass, body composition and muscle strength with age are related to the fall in circulating endogenous testosterone and estrogen concentrations. We compared various measures of the level of bioactive androgen and estrogen to which tissues are exposed.

Methods After exclusion of subjects with severe mobility problems and signs of dementia, 403 healthy men (aged 73-94 years) were randomly selected from a population based sample. Total testosterone (TT), free testosterone (FT), estrone (E1), estradiol (E2) and SHBG were determined by RIA. Levels of non-SHBG-bound testosterone (non-SHBG-T), FT (calc-FT), the TT/SHBG ratio, non-SHBG-bound E2 and free E2 were calculated. Physical characteristics of aging included muscle strength measured using dynamometry, total body bone mineral density (BMD), hip BMD and body composition, including lean mass and fat mass, measured by DEXA.

Results In this population of healthy elderly men, calc-FT, non-SHBG-T, E1, and E2 (total, free and non-SHBG-bound) decreased significantly with age. Testosterone (T, total and non-SHBG-T) was positively related with muscle strength and total body BMD (for non-SHBG-T respectively β=1.93 ± 0.52, P<0.001 and β=0.011 ± 0.002, P<0.001). An inverse association existed between T and fat mass (β=-0.53 ± 0.15, P<0.001). Non-SHBG-T and calc-FT were more strongly related to muscle strength, BMD and fat mass than TT and were also significantly related to hip BMD. E1 and E2 were both positively, independently associated with BMD (for E2 β=0.21 ± 0.08, P<0.01). Non-SHBG bound E2 was slightly stronger related to BMD than total E2. The positive relation between T and BMD, was independent of E2. E1 and E2 were not related with muscle strength or body composition.

Conclusion In summary, bioavailable testosterone, estrone, total estradiol, and bioavailable estradiol all decrease with age in healthy old men. In this cross-sectional study in healthy elderly men, non-SHBG-bound testosterone seems to be the best parameter for serum levels of bioactive testosterone, which appears to play a direct role in the various physiological changes which occur during aging. A positive relation with muscle strength and bone mineral density and a negative relation with fat mass was found. In addition, both serum estrone and estradiol seem to play a role in the age-related bone loss in elderly men, although the cross-sectional nature of the study precludes a definitive conclusion. Non-SHBG bound estradiol seems to be the best parameter of serum bioactive estradiol in describing its positive relation with bone mineral density.

INTRODUCTION

Throughout adult life, all physiological functions gradually decline. In men, part of these age-related physiological changes (loss of muscle size and strength, loss of bone, and increase in fat mass) may be related to the decrease in serum levels in bioavailable testosterone with aging (1,2). Some studies report a positive relation between estimates of serum testosterone levels and bone density in older men (1,3,4), although this has not been a universal finding (5,6). Androgen administration to older men with low plasma testosterone levels results in increases in lean body mass, bone density and/or muscle strength (7-10).

Recently it has been suggested that estrogens may play an important role in the development and maintenance of the male skeleton (11). Several studies have reported positive relations between serum estradiol concentrations and bone mineral density or bone turnover markers in men (12).

Serum testosterone as well as estradiol are mainly bound to sex-hormone binding globulin (SHBG) and albumin. It has been suggested that the fraction of testosterone bound to albumin has access to target tissues (13). It remains to be established whether total, non-SHBG-bound (albumin bound and free) or free testosterone levels are the best representation of the bioactive hormone concentrations. The same holds true for serum estradiol concentrations.

Therefore, in the present cross-sectional study of 403 independently living elderly men, we tested the hypothesis that the decreases in bone mass, body composition and muscle strength with age are related to the fall in circulating endogenous testosterone and estrogen concentrations. We compared various measures of the level of bioactive androgen to which tissues are exposed: total and free testosterone levels in serum, the total testosterone / SHBG ratio, calculated free and non-SHBG-bound testosterone levels and total testosterone adjusted for SHBG. In addition, relationships with total, non-SHBG-bound and free estradiol were compared.

METHODS

Subjects

The study is a cross-sectional, single-center study in 403 independently living men, aged 70 years and higher. Names and addresses of all male inhabitants 70 years and older were drawn from the municipal register of Zoetermeer, a medium sized town in the mid-western part of the Netherlands. 1567 Men were invited and after exclusion of subjects who did not live independently and subjects who were not physically or mentally able to visit the study center independently, eventually 403 men participated (25.7%). A total of 886 men did not respond to the mailed invitation in which it was already mentioned that subjects who did not live independently or with severe mobility problems would not be allowed to participate. The main reason not to participate among the respondents was because they were already being seen by a specialist or general

practitioner at the moment (28%), while 16% was excluded on the basis of physical (10%) or mental (6%) problems. Participants signed an informed consent. The study has been approved by the Medical Ethics Committee of the Erasmus University Hospital Rotterdam. No additional health related eligibility criteria were used. A number of participants were taking medications for chronic illnesses, like hypertension (n=96) and mild congestive heart failure (n=28). However, none of these medications did in retrospect influence the relations described in this study. Some of the illnesses, for example mild knee pain (n=79), influenced the physical characteristics measured, but in retrospect they did not change the relations between the physical characteristics, nor between the circulating hormone levels and the physical characteristics reported in this study.

Hormone measurements

Blood samples were collected in the morning after an overnight fast. The period of storage at -40□C varied from 0 till 5 months. Serum concentrations of total testosterone (TT, nmol/l), free testosterone (FT, nmol/l), and sex hormone binding globulin (SHBG, nmol/l) were all measured by radioimmunoassay using commercial kits (Diagnostic Systems Laboratories, Webster, Texas, USA). The intraassay coefficients of variation (CV) for these assays were 8.1%, 6.2% and 3.0% respectively. The interassay CVs were 10.5%, 9.7% and 4.4%. The free testosterone RIA uses an [I-125]-labeled testosterone analog which has a low affinity for SHBG and albumin. This analog competes with the unbound testosterone in the test sample for binding to specific anti-testosterone polyclonal antibodies which have been immobilized on the assay tube. This competitive binding format allows direct estimation of unlabelled free testosterone levels in unextracted samples. Furthermore, as measures of biologically active testosterone, the TT / SHBG ratios were calculated, as well as free testosterone (calc-FT, nmol/l) and non-SHBG-bound testosterone (non-SHBG-T, nmol/l, is calc-FT plus albumin bound testosterone) (Table 1) (14). In these calculations, the possible binding of other steroids to SHBG was disregarded. Finally, total testosterone adjusted for SHBG in a multiple regression analysis was used as a measure of non-SHBG-bound testosterone.

Serum concentrations of estrone (E1, nmol/l) and estradiol (E2, nmol/l) were also measured by radioimmunoassay using commercial kits (Diagnostic Systems Laboratories). The intraassay coefficients of variation (CV) were respectively 5.6% and 5.3%. The interassay CV were resp. 10.2% and 8.1%. As measures of biologically active E2, free E2 and non-SHBG-bound E2 were calculated according to the method described by Södergård (14), taking the concentration of T into account. Albumin (g/l) was measured by photometry using a commercial kit (ALB, Boehringer, Mannheim, Germany).

Table 1. Measurements and calculations for the various concentrations of testosterone and estradiol

Steroid variables	Measurements and calculations
Total Testosterone (TT, nmol/l)	Measured by RIA (Diagnostic Systems Laboratories-4100, Webster, Texas, USA)
Free Testosterone (FT, nmol/l)	Measured by RIA (Diagnostic Systems Laboratories-4900, Webster, Texas, USA)
Calculated Free Testosterone (calc-FT, nmol/l)	According to Södegård et al. (14): calc-FT = $-b + \sqrt{(b^2 + 4a[\Sigma TT])} / 2a$ $a = K^A + K^T + (K^A \times K^T)([SHBG] + [Albumin] - [\Sigma TT])$ $b = 1 + K^T[SHBG] + K^A[Albumin] - (K^A + K^T)[\Sigma TT]$
Non SHBG bound Testosterone (non-SHBG-T, nmol/l)	According to Södegård et al. (14): non-SHBG-T= $(K^A \times [Albumin] \times [calc\text{-}FT] / 1 + K^A \times [calc\text{-}FT]) + [calc\text{-}FT]$
TT / SHBG ratio	Measured Total Testosterone / Measured Sex Hormone Binding Globulin ratio
TT adj. SHBG, nmol/l	Measured Total Testosterone Adjusted for measured SHBG in a multiple regression analysis
Estradiol (nmol/l)	Measured by RIA (Diagnostic Systems Laboratories-4400, Webster, Texas, USA)
Calculated Free Estradiol (fE2, nmol/l)	According to Södegård et al. (14): $fE2 = -b + \sqrt{(b^2 + 4ac)} / 2a$ $a = (K^A \times [Albumin] + 1)(K^E)$ $b = ([\Sigma E2] \times K^E) - (K^{AE} \times [Albumin] + 1)(1 + K^T[SHBG] \times calc\text{-}FT) - (K^{TE} \times [SHBG])$ $c = [\Sigma E2] \times (1 + K^T[SHBG] \times calc\text{-}FT)$
Non SHBG bound Estradiol (non-SHBG-E2, nmol/l)	According to Södegård et al. (14): non-SHBG-E2 = $[\Sigma E2] - (K^{TE} \times [SHBG] \times fE2 / (1 + K^{TE} \times fE2 + K^T[SHBG] \times calc\text{-}FT)$

K^A= association constant for binding of testosterone to Albumin

K^T= association constant for binding of testosterone to SHBG

K^{AE}= association constant for binding of estradiol to Albumin

K^E= association constant for binding of estradiol to SHBG

Physical characteristics of aging

Muscle Strength

Isometric grip strength (IGS) was measured using an adjustable hand held dynamometer (JAMAR dynamometer) at the non-dominant hand (15). Each test was repeated three times and the average was used in the analyses. Leg or knee extensor strength (LES) was measured as described previously (16,17) using the Hoggan MicroFET hand held dynamometer. To obtain one main outcome measurement for leg

extensor strength, "maximum leg extensor strength" (maxLES) was defined as the maximum strength for the right or left leg, whichever is largest, in a position of 120° extension. Statistical analyses were based on the physical unit momentum (Nm), obtained by multiplying the maximum strength (in Newton) and the distance of the dynamometer to the knee joint (in meters).

Bone mineral density and body composition

Total body bone mineral density (TBBMD) was measured using dual energy x-ray absorptiometry (DEXA, Lunar, Madison, WI), as were hip bone mineral densities at the femoral neck, trochanter and Ward's triangle (18). In addition, total and trunk lean body mass and fat mass were measured (19,20). Quality assurance for DEXA, including calibration was performed every morning, using the standard provided by the manufacturer.

Height and weight were measured in standing position without shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. The waist circumference was measured at the level of the umbilicus and the hip circumference was measured at the level of the greater trochanter. The average of two readings was used in the analyses. Waist / hip ratio (WHR), which represents a measure of upper body adiposity, was calculated from these two measurements.

Data analyses

Results were expressed, unless otherwise stated, as mean and standard deviation with the interquartile (IQ) range. Comparisons between groups were made by using Student's *t*-test. Differences were given together with the 95% confidence-interval (CI). Relations between variables were assessed using linear regression for continuous variables and logistic regression for binary variables, and described as the linear regression coefficient (β) and its standard error (S.E.). Multiple regression analysis was used to adjust for age and BMI, as well as to assess the contribution of different independent variables to the dependent variable. To assess the contribution of different variables on the dependent parameter, we used standardized regression, described as the standardized regression coefficient (B). Standardized regression coefficients are regression coefficients normalized by the ratio of the standard deviation of the dependent variable. Correlations between variables were assessed by using Pearson's correlation coefficient *r*. All analyses were done using STATA Statistical Software: Release 5.0: Stata Corporation.

RESULTS

Descriptive data of the physical characteristics, as well as age and body mass index (BMI) are shown in Table 2. Muscle strength, bone mineral density (BMD), lean body mass (LBM), and fat mass all decreased with age in our study group (Table 3). Because virtually all parameters were related with age, all further analyses were done after

adjustment for age. Descriptive data of the hormone measurements are shown in Table 4.

Relations of the hormone concentrations with age

The relations of the hormone measurements with age are shown in Table 5. In our population all testosterone (T) measurements, except total testosterone (TT), significantly decreased with age. The relation between non-SHBG-bound T and age is illustrated in Figure 1. Serum SHBG and percentages of SHBG-bound T and estradiol (E2) increased with age. Estrone (E1) and total E2 concentrations were inversely related with age. Calculated free E2 (fE2) and calculated non-SHBG-bound E2 (non-SHBG-E2) decreased relatively more with age compared with total estradiol (E2) (standardized regression coefficients (B) were respectively -0.15, -0.15 and -0.12).

Table 2. Descriptive Data of the study population

	Mean	± SD	Interquartile range	
Age (yr)	77.8	3.58	75	80
Body Mass Index	25.5	3.0	23.3	27.3
Isometric Grip Strength (kg)	34.3	6.9	30.0	38.7
Maximum Leg Extensor Strength (Nm)	103.2	20.9	89.43	117.12
Total Fat Mass (kg)	21.2	6.4	17.4	24.5
Trunk Fat Mass (kg)	10.6	2.6	9.0	12.4
Total Lean Mass (kg)	51.7	5.6	47.8	55.5
Total Body Bone Mineral Density (g/cm^2)	1.17	0.10	1.11	1.23
Femoral Neck Bone Mineral Density (g/cm^2)	0.88	0.14	0.78	0.97
Femoral Ward Bone Mineral Density (g/cm^2)	0.72	0.16	0.60	0.82
Femoral Trochanter Bone Mineral Density (g/cm^2)	0.85	0.15	0.76	0.94

Table 3. Relationship between physical characteristics and age

	Age (year)		
	β	± SE	P-value
Isometric Grip Strength (kg)	-0.53	0.09	<0.001
Maximum Leg Extensor Strength (Nm)	-1.30	0.28	<0.001
Total Fat Mass (kg)	-0.18	0.08	0.02
Trunk Fat Mass (kg)	-0.10	0.04	0.007
Total Lean Mass (kg)	-0.38	0.08	<0.001
Total Body Bone Mineral Density (g/cm^2)	-0.003	0.001	0.006
Femoral Neck Bone Mineral Density (g/cm^2)	-0.002	0.002	0.25
Femoral Ward Bone Mineral Density (g/cm^2)	-0.003	0.002	0.17
Femoral Trochanter Bone Mineral Density (g/cm^2)	-0.004	0.002	0.04

Table 4. Summarized values of hormone levels of the 403 men

	Mean ± S.D.		Interquartile range	
Total Testosterone (TT, nmol/l)	8.83	2.98	7.19	10.66
Free Testosterone (FT, nmol/l)	0.03	0.01	0.024	0.038
Sex Hormone Binding Globulin (nmol/l)	31.5	14.4	23.18	37.46
Calculated FT (nmol/l)	0.19	0.06	0.16	0.23
Non-SHBG-bound Testosterone (nmol/l)	5.68	1.89	4.62	6.76
Percentage SHBG bound T (%)	35.0	9.51	29.2	41.1
Percentage albumin bound T (%)	62.8	9.23	56.9	68.6
Percentage free T (%)	2.21	0.31	2.03	2.39
TT/SHBG ratio	0.32	0.15	0.23	0.39
Estrone (nmol/l)	0.102	0.039	0.075	0.126
Estradiol (E2, nmol/l)	0.098	0.058	0.059	0.126
Calculated Free E2 (pmol/l)	2.5	1.4	1.5	3.2
Non-SHBG-bound E2 (nmol/l)	0.077	0.045	0.045	0.100
Percentage SHBG bound E2 (%)	21.9	7.40	17.2	26.1
Percentage albumin bound E2 (%)	75.5	7.21	71.4	80.2
Percentage free E2 (%)	2.56	0.24	2.42	2.72

Table 5. Relationship between age and hormone levels

	Age (yr)		
	β	± S.E.	P
Total Testosterone (TT, nmol/l)	-0.04	0.04	0.37
Free Testosterone (FT, nmol/l)	-0.001	0.0001	<0.001
Sex Hormone Binding Globulin (nmol/l)	0.92	0.20	<0.001
Calculated FT (nmol/l)	-0.002	0.001	0.02
Non-SHBG-bound Testosterone (nmol/l)	-0.07	0.03	0.01
Percentage SHBG bound T (%)	0.66	0.13	<0.001
Percentage albumin bound T (%)	-0.64	0.12	<0.001
Percentage free T (%)	-0.029	0.004	<0.001
TT/SHBG ratio	-0.009	0.002	<0.001
Estrone (nmol/l)	-0.005	0.0005	<0.001
Estradiol (E2, nmol/l)	-0.002	0.0008	0.01
Calculated Free E2 (pmol/l)	-0.060	0.020	0.003
Non-SHBG-bound E2 (nmol/l)	-0.002	0.001	0.002
Percentage SHBG bound E2 (%)	0.51	0.10	<0.001
Percentage albumin bound E2 (%)	-0.50	0.10	<0.001
Percentage free E2 (%)	-0.013	0.003	<0.001

β = coefficient of linear regression represents changes in unit per year. E.g.: Non-SHBG-T decreases 0.07 nmol/l per year.

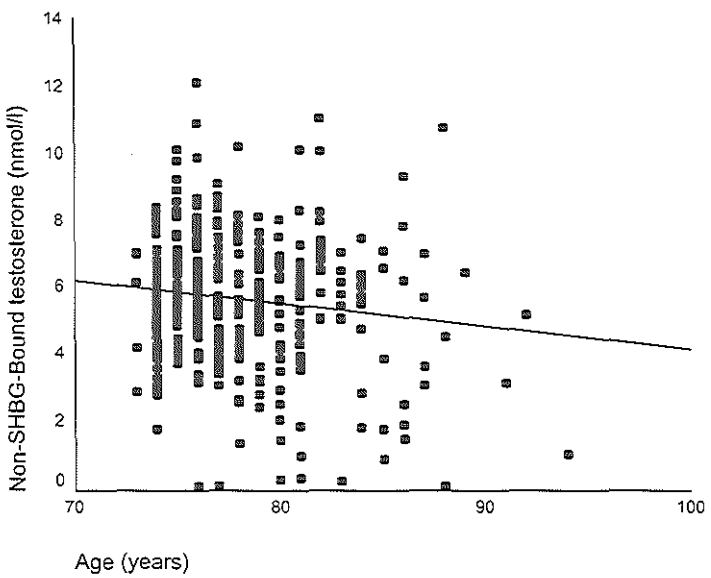

Figure 1. Relationship between non-SHBG-bound testosterone and age. Coefficient denotes the linear regression coefficient. F.e. non-SHBG-bound testosterone decreases 0.07 nmol/l per year.

Relations between physical characteristics

Lean mass, fat mass and maxLES were all positively, independently related with TBBMD (respectively; β=0.0060 ± 0.0008 kg/(g/cm^2), P<0.001, β=0.0063 ± 0.0008 kg/(g/cm^2), P<0.001 and β=0.0011 ± 0.0002 Nm/(g/cm^2), P<0.001). Lean mass and maxLES were both positively associated (β=0.12 ± 0.01 Nm/kg, P<0.001).

Relations between hormone concentrations

Mean values of measured FT were significantly lower than calc-FT (difference 0.16 ± 0.003 nmol/l, CI= 0.156-0.166, P<0.001). Results of the different T measurements correlated well with each other (Table 6). Correlations between serum levels of T, E1 and E2 are shown in Table 7. E1 and E2 were positively related with each other, and with the various measures of testosterone.

Table 6. Age adjusted relations between different measures of testosterone concentrations

	TT (nmol/l)		TT/SHBG ratio		Calculated FT (nmol/l)	
	β ± S.E.	*r*	β ± S.E.	*r*	β ± S.E.	*r*
Meas. FT (nmol/l)	181.1 ± 7.19	0.78[1]	0.03 ± 0.004	0.39[1]	0.15 ± 0.007	0.75[1]
Calc. FT (nmol/l)	42.80 ± 1.00	0.91[1]	0.29 ± 0.02	0.68[1]		
Non-SHBG-T (nmol/l)	1.42 ± 0.04	0.89[1]	8.98 ± 0.45	0.71[1]		
TT/SHBG ratio	8.04 ± 0.92	0.40[1]				

[1]= P-value <0.001, TT = Total Testosterone, FT = Free Testosterone, Non-SHBG-T = calculated non SHBG bound testosterone

Table 7. Age adjusted relations between serum testosterone and estrogens levels

	TT	Non-SHBG-T	calc FT	E1	E2	Free E2
	r	*r*	*r*	*r*	*r*	*r*
Estrone (E1)	0.28[1]	0.28[1]	0.29[1]			
Estradiol (E2)	0.17[1]	0.18[1]	0.14[2]	0.24[1]		
Free E2	0.14[2]	0.20[1]	0.16[2]	0.24[1]	0.98[1]	
Non-SHBG-E2	0.13[2]	0.20[1]	0.18[1]	0.24[1]	0.99[1]	0.995[1]

r denotes correlation coefficient [1]= P-value <0.001, [2]= P-value <0.01

TT = Total Testosterone, calc FT = calculated free testosterone, Non-SHBG-T/E2 = calculated non SHBG bound testosterone / estradiol

Relation between testosterone concentrations and physical characteristics

Because BMI was significantly related to levels of T, maxLES, IGS and BMD, all analyses including these parameters were done after adjustment for BMI.

All T measurements were positively related to TBBMD after adjustment for age (Table 8). Calc-FT, non-SHBG-T, the TT/SHBG ratio and TT adjusted for SHBG were also significantly positively related to all regions of proximal femur BMD. As an example, the relationships between the different T measures and BMD of the Ward's triangle are shown in Table 8.

None of the T measurements, with the exception of the TT / SHBG ratio, was related with lean mass (not shown). Fat mass was negatively associated with all T measurements, except with the TT / SHBG ratio (Table 8).

All measurements for T were positively related with maxLES (Table 8) and with IGS and after adjustment for age (with the exception that FT was not related with IGS). T was independently related to both muscle strength and BMD.

The SHBG-bound fraction of T was not related to any of the physical characteristics, with the exception of fat mass (β=-1.03 ± 0.18, P<0.001). The strength of the relations of the albumin bound fraction of T to the physical characteristics was equal to those for non-SHBG-T.

Serum SHBG concentrations were inversely related to lean mass (β=-0.07 ± 0.02 kg/(nmol/l), P<0.001), fat mass (β=-0.08 ± 0.02 kg/(nmol/l), P<0.001) and BMD (e.g. TBBMD; β=-0.001 ± 0.0003 (g/cm2)/(nmol/l), P=0.01).

Relations between estrogens and physical characteristics

Serum E1 and E2 concentrations were not related to muscle strength. In this elderly male population, serum E1 levels were positively associated with TBBMD (β=0.36 ± 0.12 (g/cm^2)/(nmol/l), P=0.004, age adjusted). Serum E2 concentrations (total, free and non-SHBG bound) were positively related to TBBMD as well as to hip BMD (Table 9). Serum E1 contributed slightly more to the variation in TBBMD, as shown by the standardized regression coefficients (respectively 0.10 for E2 and 0.13 for E1). To investigate whether the above described effect of T on BMD might be mediated through its aromatization to estrogens, a multiple regression analysis was performed, including BMD as the dependent variable and E2 and T as the independent variables. E2 and TT remained independently related to TBBMD, and contributed equally to the variation of TBBMD, as shown by the standardized regression coefficients (both 0.11). Again, serum E1 contributed to a slightly greater variation in TBBMD compared to TT (standardized regression coefficients respectively 0.12 and 0.10). Further, with regard to hip BMD, E2 and non-SHBG-T remained independently, significantly related to BMD. Similar results were obtained when non-SHBG-E2 was the independent variable.

Measures of E1 and E2 were not related to body composition: neither to lean mass, nor to total or trunk fat mass. Nor were they related to fat mass after adjustment for T. A weak positive association was found between E1 and waist-hip ratio (β=0.13 ± 0.07 /(nmol/l), P=0.05).

Table 8. Relations between testosterone measurements and physical characteristics of successful aging

	Maximun Leg Extensor Strength (Nm)			Total Body Bone Mineral Density (g/cm2)			Ward Bone Mineral Density (g/cm2)			Fat Mass (kg)		
	β	S.E.	B	β	S.E.	B	β	S.E.	B	β	S.E.	B
Total Testosterone (TT, nmol/l)	0.99	0.33	0.14[2]	0.00	0.00	0.13[2]	0.005	0.003	0.08[4]	-0.49	0.09	-0.13[1]
Free Testosterone (FT, nmol/l)	151.	76.6	0.10[3]	0.69	0.35	0.09[3]	0.88	0.63	0.07[4]	-86.0	21.8	-0.10[1]
Calculated FT (nmol/l)	55.5	15.4	0.17[1]	0.28	0.07	0.18[1]	0.35	0.13	0.14[2]	-16.6	4.5	-0.13[1]
Non-SHBG-T (nmol/l)	1.93	0.52	0.17[1]	0.01	0.00	0.20[1]	0.012	0.004	0.14[2]	-0.53	0.15	-0.13[1]
TT / SHBG ratio	25.5	6.56	0.18[1]	0.14	0.03	0.22[1]	0.14	0.05	0.13[2]	-1.77	1.93	-0.12[4]
TT adjusted for SHBG (nmol/l)	1.11	0.34	0.16[1]	0.00	0.00	0.16[1]	0.007	0.003	0.12[3]	-0.42	0.10	-0.14[1]

β denotes coefficient of linear regression, B denotes standardized linear regression coefficient
Non-SHBG-T denotes non SHBG bound testosterone
All analyses were done after adjustment for age and BMI.
[1] P≤0.001, [2] P≤0.01, [3] P≤0.05, [4] P>0.05

Table 9. Relation between serum estradiol concentrations and bone mineral density (BMD)

	Estradiol (E2, nmol/l)		Free E2 (pmol/l)		Non-SHBG-E2 (nmol/l)	
	β ± S.E.	B	β ± S.E.	B	β ± S.E.	B
Total body BMD (g/cm²)	0.21 ± 0.08	0.13[2]	0.009 ± 0.003	0.14[2]	0.31 ± 0.10	0.14[2]
Femur Neck BMD (g/cm²)	0.26 ± 0.12	0.11[3]	0.011 ± 0.005	0.11[3]	0.36 ± 0.16	0.11[3]
Femur Ward BMD (g/cm²)	0.29 ± 0.14	0.11[3]	0.013 ± 0.006	0.11[3]	0.42 ± 0.18	0.12[3]
Femur Trochanter BMD (g/cm²)	0.32 ± 0.12	0.12[2]	0.013 ± 0.005	0.13[2]	0.41 ± 0.15	0.13[2]

β denotes coefficient of linear regression. B denotes standardized linear regression coefficient
All analyses were done after adjustment for age and BMI. [1] P≤0.001, [2] P≤0.01, [3] P≤0.05

DISCUSSION

In this population, circulating non-SHBG-bound and free testosterone (T), estrone (E1), and estradiol (E2) (total, free and non-SHBG-bound) levels all declined with age. Serum total testosterone (TT) did not change with age, while the SHBG bound fraction of both T and E2 increased with age. Serum concentrations of the non-SHBG-bound T were most strongly related to several physical characteristics of aging; positively with muscle strength and bone mineral density (BMD), and inversely with fat mass. Serum E1 and E2 concentrations were positively associated with BMD, but not with muscle strength. Free and non-SHBG-bound E2 were more strongly related to BMD than total E2.

Serum T concentrations as found in this study seem to be relatively low. Furthermore, calc-FT concentrations were significantly higher than measured FT. It has to be questioned if the FT assay provides a good method to measure the biologically active fraction of circulating T. Our findings that FT (both measured and calculated), non-SHBG-T and the TT/SHBG ratio decrease with increasing age, are in agreement with previous studies (2,21,22). Total testosterone (TT) levels did not decrease with age, but TT levels in this population were below the normal range for middle aged adults, suggesting that T levels had already declined in comparison with younger men. To our knowledge, no reference values for percentage of SHBG bound, albumin bound and free fractions of testosterone exist in the elderly. The decrease of bioavailable T is probably due to the increase of SHBG-bound T with age, in combination to the decrease of the albumin bound fraction to a similar extent.

Until recently not much attention has been paid to the role of estrogens in elderly men. The studies which have been reported so far, show no change of total E2 levels with age in men (23-25), or a decrease of E2 levels only at old age (26). In the present study serum total E2 was inversely associated with age. Bioavailable and free E2 concentrations decreased with age to an even greater extent than total E2. Two earlier studies also reported a decrease of bioavailable E2 (12,25). We studied, however, an older

and larger population compared to these studies. As with T, the SHBG bound fraction of serum E2 increased with age, while the albumin fraction decreased. In addition, serum E1 concentrations decreased with age.

A number of clinical problems prevalent in older men may be related to androgen deficiency, including reduced muscle strength (27,28), changes in body composition and loss of bone mineral density (1,3,4). It should be of interest to know whether the decrease in bone mass with age is causally related with the decrease in lean mass, and/or in serum testosterone or estradiol concentrations. Also, it would be important to investigate whether the fall in lean mass with age is caused by the decrease in muscle strength, and/or serum testosterone or estradiol concentrations. Finally, also the reverse could be the case, in which the decrease in muscle strength with age, is caused by the fall in lean mass, and/or serum testosterone levels or other factors. Unfortunately, the cross-sectional nature of our study does not allow a differentiation between cause and effect. All the factors are statistically related, making it impossible to differentiate which factor explains another. Therefore no conclusions can be drawn concerning the physiological pathway(s). For example, also physical activity *per se* might be a factor contributing to the relationships found. A longitudinal study is necessary to answer these questions. From the results of our study, we can, however, confirm previous findings, that serum testosterone was, independent of age, positively related to both IGS and maxLES. In addition, we found a positive, age independent, relation between estimates of serum testosterone levels and bone density. Further, in agreement with previous findings, testosterone was inversely associated with fat mass, but not with lean body mass (1). Serum non-SHBG-bound T concentrations are not only related to TBBMD, but also to all regions of the proximal femur BMD.

In this study we established whether free T, the non-SHBG-bound fraction of T or TT measured in plasma represent the bioactive hormone best. Pardridge suggested that albumin-bound T is available for uptake by most tissues, while SHBG-bound T is not (13). Considering the fact that non-SHBG-T as well as the albumin bound fraction were strongly related to the physical characteristics of aging, it is possible that the albumin bound fraction of T is available for uptake by tissues and can exert biological effects. Furthermore, in agreement with the suggestion of Pardridge, SHBG-bound T was not related to any physical characteristic. Serum non-SHBG-T concentrations were stronger related to the physical characteristics than TT, as shown by the standardized regression coefficients, and slightly stronger than serum calc-FT concentrations. In addition, non-SHBG-T levels were related to proximal femur BMD, while TT levels were not.

There was a considerable difference in the relationships between non-SHBG-bound T calculated according to the method described by Södergård et al. on the TT/SHBG ratio as a measurement of non-SHBG-bound T on the one hand and the physical characteristics on the other hand. The TT/SHBG ratio probably reflects in part the non-testosterone dependent inverse association of SHBG with these characteristics (demonstrated in the relation with lean mass, fat mass and BMD). Furthermore, use of the ratio obfuscates the absolute levels of the components of the quotient. TT adjusted for SHBG as a measure of non-SHBG-bound T, is probably a better parameter than

TT/SHBG, because the inverse associations of SHBG are not taken into account. However, as judged by the standardized regression coefficients, it shows no clear advantage compared to non-SHBG-T. Thus, the calculated bioavailable T concentration according the method described by Södergård et al. appears to be an easy, inexpensive and informative measure in representing the bioactive fraction of circulating T.

Most of the E2 and about 20 % of E1 produced in normal men is formed by extraglandular aromatization of circulating androgens. A smaller part of the circulating E2 is derived from a direct secretion by the testicles (29). Most of the E1 in elderly men is produced by the adrenals. Recently it has been demonstrated that estrogens play an important role in maintaining bone mineral density in healthy older men (5,30). Trabecular bone is generally thought to be more responsive to gonadal steroids than cortical bone. However, we did not measure spinal BMD, as degenerative arthritis influences the outcome of measurements too strongly. In our population serum total E2 was independent of age, strongly positively related to BMD at all sites measured. Serum non-SHBG-bound E2 was slightly stronger related to BMD at all sites measured compared to total E2, as shown by the standardized regression coefficients. This suggests that the non-SHBG-bound fraction of E2 is the best representation of bioactive hormone, although to a lesser extent than is the case for testosterone, possibly because E2 is more dissociable from SHBG than T. These findings are in agreement with those described by Khosla et al. (12). Also serum E1 concentrations were related to bone density, although to TBBMD only. However, this relation was independent of E2 and T and contributed to an even greater extent to the variation in TBBMD than E2 or T levels. This might imply that E1 has an effect on BMD directly, or that it is locally converted to E2. Serum T and E2 both contribute equally to the variation in BMD, suggesting that the effect of T on bone is not only attributed to the aromatization to estrogens (31).

Although it is generally accepted that estrogens and fat mass, especially trunk fat, are strongly related in postmenopausal women (32), we did not find a significant relation between E1 or E2 and (trunk) fat mass in these elderly men, before or after adjustment for testosterone. This confirms other findings that aromatase activity is also present in other tissues apart from fat (33-36).

In summary, together with a decline in muscle strength, bone density and body composition, bioavailable testosterone, estrone, total estradiol, and bioavailable estradiol all decrease with age in healthy old men. In this cross-sectional study in healthy elderly men, non-SHBG-bound testosterone seems to be the best parameter for serum levels of bioactive testosterone, which appears to play a direct role in the various physiological changes which occur during aging through its positive relation with muscle strength and bone mineral density and its negative relation with fat mass. In addition, both serum estrone and estradiol seem to play a role in the age-related bone loss in elderly men, although the cross-sectional nature of this study precludes definitive conclusion. Non-SHBG bound estradiol seems to be the best parameter for serum levels of bioactive estradiol in describing its positive relation with bone mineral density.

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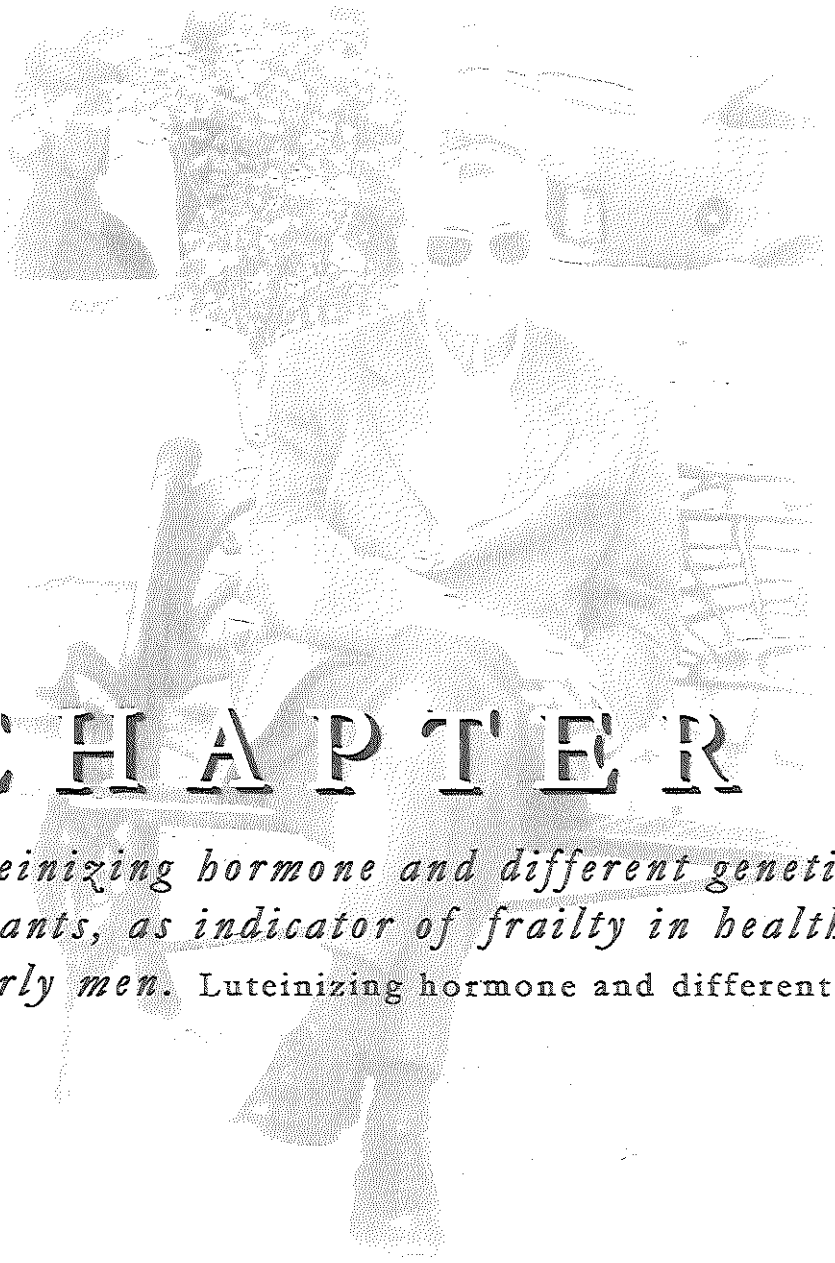

CHAPTER 4

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ABSTRACT

Objective We investigated the possible clinical correlates between the serum LH concentration and characteristics of frailty, and determined the presence and concentration of a genetic LH variant, in an independently living population of elderly men.

Methods After exclusion of subjects with severe mobility problems and signs of dementia, 403 healthy men (aged 73-94 years) were randomly selected from a population based sample. Total testosterone (TT), SHBG and leptin were determined by RIA. Non-SHBG bound testosterone (non-SHBG-T) was calculated. Luteinizing hormone (LH) and the presence of the genetic LH variant were measured using immunofluorometric assays. The characteristics of frailty were leg extensor strength (maxLES) using dynamometry, bone mineral density (BMD) of total body and proximal femur and body composition, including lean mass and fat mass, measured by dual energy X-ray absorptiometry. Disability was further assessed by the Modified Health Assessment Questionnaire (MHAQ) and by a measure of physical performance (PPS).

Results LH significantly increased with age, and inversely correlated with TT and non-SHBG-T. LH was inversely related to muscle strength and lean mass and both relations were independent of testosterone. LH was positively related to self-reported disability (MHAQ). 12.5% of the study population was heterozygous for the LH variant allele. Testosterone levels and the degree of frailty were not different in the wild-type LH group compared with the heterozygote LH variant group. However, the proportion of heterozygote LH variants was significantly higher in those subjects with TT or non-SHBG-T concentrations in the lowest 10% of our study population and LH concentrations >10 IU/l. A significant positive relation between LH and fat mass as well as leptin was only present in the heterozygote LH variant group.

Conclusions Serum LH levels increases with age in independently living elderly men and correlates inversely with a variety of indicators of frailty. The observed relation between LH and frailty, independent of T, suggests that LH reflect serum androgen activity in a different way than T, possibly reflecting more closely the combined feedback effect of estrogen and androgen. As a relatively higher proportion of subjects heterozygous for the LH variant allele had high LH and low T levels, the two LH forms may differ in biological response, in favor of wild-type LH.

INTRODUCTION

Physical frailty in the elderly is defined as "a state of reduced physiological reserves associated with increased susceptibility to disability" (1). Age-related disability is characterized by generalized weakness, impaired mobility and balance, and poor endurance. Clinical correlates of physical frailty include falls, fractures, impairment in activities of daily living, and loss of independence: falls contribute to 40% of admissions to nursing homes (2). Falls are significantly associated with slow gait, poor physical performance and lack of muscle strength (3-5).

In a previous study we demonstrated that low testosterone (T) levels in a population of healthy elderly men were associated with reduced muscle strength and bone mineral density and increased fat mass (unpublished data). Since a negative feedback relationship exists between T and LH, also the circulating LH level may serve as an indicator of frailty.

Recently a common variant form of LH was detected in apparently healthy individuals, caused by point mutation-based substitutions of two amino acids ($Trp^{8}Arg$ and $Ile^{15}Thr$) in the LHβ subunit (6-8). Suggestions have been made that the in vivo bioactivity of the LH variant is lower than that of the wild-type hormone, due to its shorter circulatory half-time (9,10). Raivio et al.(10) suggested that the occurrence of the variant LH may be a factor contributing to delayed pubertal tempo in otherwise healthy boys. No data are available yet describing the presence and bioactivity of this LH variant in elderly populations.

We investigated serum LH levels in relation to characteristics of frailty and determined the presence and concentration of the genetic LH variant in an independently living population of elderly men. The purpose was to find out whether any of the variable alterations in the pituitary-testicular function with aging could be related to the occurrence of a biologically dissimilar variant form of LH.

METHODS

Subjects

A group of 403 independently living men, aged 73 or above, participated in this study. Participants were recruited by letters of invitation, which were sent to the oldest male inhabitants of Zoetermeer, a medium sized town in the mid-western part of the Netherlands. All participants provided informed consent, and the Frail Old Men study was approved by the Medical Ethics Committee of the Erasmus University Hospital Rotterdam. Participants were judged sufficiently healthy to participate in the study when they were physically and mentally able to visit the study center independently. No additional health related eligibility criteria were used.

Hormone measurements

Blood samples were collected in the morning after an overnight fast. Serum concentrations of total testosterone (TT, nmol/l), sex hormone binding globulin (SHBG, nmol/l) were all measured by radioimmunoassay using commercial kits (Diagnostic System

Laboratories, Webster, Texas, USA). The intraassay coefficients of variation (CV) were respectively 8.1%, 6.2% and 3.0%. The interassay CVs were resp. 10.5%, 9.7% and 4.4%. The Free Testosterone RIA uses an [^{125}I]-labeled testosterone analog which has low affinity for SHBG and albumin. Non SHBG bound testosterone (non-SHBG-T, nmol/l) was calculated according to a method described by Södergård et al. (11). Leptin (µg/l) was also measured by radioimmunoassay (Lilly Research Laboratories, Giessen, Germany). Albumin (g/l) was measured by photometry using a commercial kit (ALB, Boehringer, Mannheim, Germany).

Luteinizing hormone (LH, IU/l) was measured by an immunofluorimetric assay (Delfia, Wallac Oy, Turku, Finland). The LH variant was recognized after calculation of the ratio of the results of two LH assays. The Delfia method for LH (LHspec), which uses two LH β-subunit-specific monoclonal antibodies (mAb) (12,13) served as a reference method (assay 2). In the other assay (assay 1), the capture mAb recognizes a conformational epitope present in the wild-type α/β LH dimer but not in the variant form of LH or the free subunits, and the detection mAb recognizes an epitope in the α subunit (6). The ratio of LH values measured by the two assays (assay 1/ assay 2) was used to assess the variant or wild-type LH status. Three separate categories of this ratio were obtained: [1] normal ratio (>0.9), [2] low ratio (0.2 to 0.9), and [3] zero ratio (<0.15). A normal ratio individual has two wild-type LH-alleles; a low ratio individual is heterozygous for the LH variant allele; and a zero ratio individual is homozygous for the variant LHβ gene, as confirmed by DNA analysis (9, 14, 15). The sensitivity of the two immunofluorimetric assays was 0.05 IU/l, and the intra-assay and interassay coefficients of variation were < 4% and 5%, respectively, at LH concentrations at and above the lowest standard concentration (0.6 IU/l of the World Health Organization International Reference Preparation 80/552).

Measures of Muscle Strength

Isometric grip strength (IGS) was measured using an adjustable hand held dynamometer (JAMAR dynamometer) at the non-dominant hand (16). Each test was repeated three times and the average was used in the analyses. Leg or knee extensor strength (LES) was measured as described previously (17,18) using the Hoggan MicroFET hand held dynamometer. To obtain one main outcome measurement for leg extensor strength, "maximum leg extensor strength" (maxLES) was defined as the maximum strength for the right or left leg, whichever is largest, in a position of 120° extension. Statistical analyses were based on the physical unit momentum (Nm), obtained by multiplying the maximum strength (in Newton) and the distance of the dynamometer to the knee joint (in meters).

Bone mineral density and body composition measurements

Total body bone mineral density (TBBMD) was measured using dual energy x-ray absorptiometry (DEXA, Lunar, Madison, WI), as were hip bone mineral densities at the femoral neck, trochanter and Ward's triangle. In addition, total and trunk lean body mass and fat mass were measured (19). Quality assurance for DEXA, including calibration was performed routinely every morning, using the standard provided by the manufacturer.

Height and weight were measured in standing position without shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in

meters.

Physical Performance

Lower extremity function, or physical performance score (PPS), was assessed as described by Guralnik et al. (20), including measurements of standing balance, walking speed and ability to rise from a chair. Three tests of standing balance were considered in hierarchical difficulty in assigning a single score of 0 to 4 for standing balance. For the 8-foot walk and repeated chair stands, those who could not complete the task were assigned a score of 0. Those completing the task were assigned scores of 1 to 4, corresponding to the quartiles of time needed to complete the task, with the fastest times scoring as 4. A summary performance scale was created by summing the category scores for the walking, chairstand, and balance test.

Satisfaction in performing activities of daily living (ADL) was assessed by using a self-administered questionnaire from the Stanford Modified Health Assessment Questionnaire (MHAQ) as described by Pincus et al (21), in which high score means low ability. The final score of the MHAQ was not normally distributed. Analyses were done after logarithmic transformation.

Data analyses

Results were expressed, unless otherwise stated, as mean and standard deviation with the interquartile (IQ) range. Relations between variables were assessed using linear regression for continuous variables and logistic regression for binary variables, and described as the linear regression coefficient (β) and its S.E.. Multiple regression analysis was used to adjust for age and BMI, as well as to assess the contribution of different independent variables to the dependent variable. Correlations between variables were assessed by using Pearson's correlation coefficient r. Pearson chi-square test was used to assess differences of variables between groups. If not mentioned otherwise all analyses were done after adjustment for age.

RESULTS

Descriptive data of all parameters measured are shown in Table 1. Their relations with age are shown in Table 2. LH was significantly positively related with age and SHBG (Table 3). An inverse relation existed between LH and the different testosterone (T) measurements (Figure 1 and Table 3). Albumin and leptin levels were not related with LH.

Testosterone and frailty

Testosterone (T) was independently related to muscle strength, bone mineral density and fat mass. Linear regression coefficients for relations between non SHBG bound T (non-SHBG-T) and maximum leg extensor strength (maxLES), respectively, total body bone mineral density (TBBMD) were $\beta = 1.93 \pm 0.53$ and $\beta = 0.01 \pm 0.002$ ($P<0.001$). T was not related to lean mass or the physical performance score.

Table 1. Descriptive Data of the study population (n=403)

	Mean	± SD	Interquartile range	
Age (yr)	77.8	3.58	75	80
BMI	25.5	3.04	23.32	27.25
Total Testosterone (nmol/l)	8.83	2.98	7.19	10.66
SHBG (nmol/l)	31.5	14.4	23.2	37.5
Non SHBG bound T (nmol/l)	5.68	1.89	4.62	6.76
Luteinizing Hormone (IU/l)	9.38	8.41	4.57	11.15
Leptin (µg/l)	5.35	4.21	2.45	6.90
Albumin (g/l)	45.6	2.76	43.8	47.5
IGS (kg)	34.3	6.9	30.0	38.7
MaxLES (Nm)	103.2	20.9	89.4	117.1
Total Fat Mass (kg)	21.2	6.4	17.4	24.5
Total Lean Mass (kg)	51.7	5.6	47.8	55.5
Total Body BMD (g/cm^2)	1.17	0.10	1.11	1.23
Femoral Neck BMD (g/cm^2)	0.88	0.14	0.78	0.97
Femoral Ward BMD (g/cm^2)	0.72	0.16	0.60	0.82
Femoral Trochanter BMD (g/cm^2)	0.85	0.15	0.76	0.94
Physical Performance (points)	8.45	2.43	7	10
MHAQ (points)	10.7	4.30	8	12

IGS denotes Isometric Grip Strength. MaxLES denotes Maximum Leg Extensor Strength. MHAQ denotes Modified Health Assessment Questionnaire. Normative values for healthy young men are 9.72 - 30.54 nmol/l for Total Testosterone, 10 - 55 nmol/l for SHBG, and 3.6 - 17.1 IU/l for Luteinizing Hormone.

Table 2: Relationship between age and the hormones and the characteristics of Frailty

	Age (yr)		
	β	± S.E.	P-value
Luteinizing Hormone (IU/l)	0.06	± 0.01	<0.001
Total Testosterone (nmol/l)	-0.04	± 0.04	0.37
SHBG (nmol/l)	0.92	± 0.20	<0.001
Non SHBG bound Testosterone (nmol/l)	-0.07	± 0.03	0.01
Leptin (µg/l)	-0.02	± 0.01	0.04
Albumin (g/l)	-0.07	± 0.04	0.06

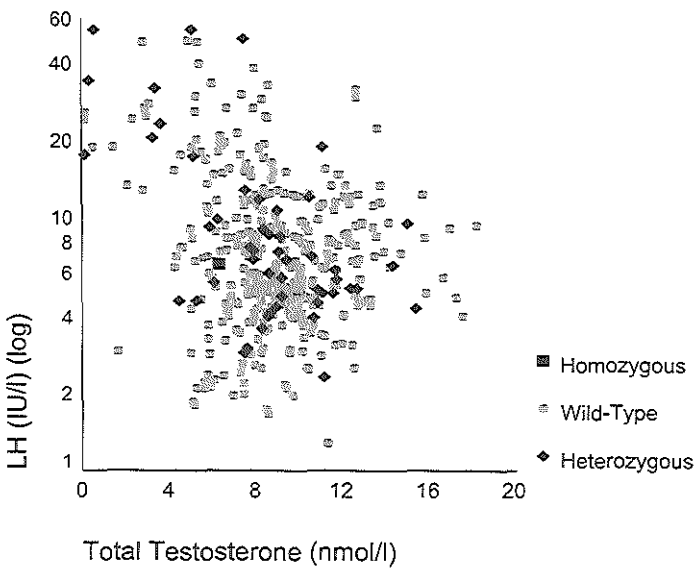
β coefficient denotes changes in unit per year. For example: LH increases with 0.06 IU/l per year

Table 3. Associations of LH with hormones, SHBG, albumin and characteristics of frailty.

	LH (IU/l)		(n=403)
	β	± S.E.	P
Hormones (dependent variables)			
Total Testosterone (nmol/l)	-0.12	0.02	<0.001
SHBG (nmol/l)	0.31	0.09	<0.001
Non SHBG-Testosterone (nmol/l)	-0.09	0.01	<0.001
Leptin (log) (μg/l)	0.002	0.005	0.63
Albumin (g/l)	-0.002	0.02	0.91
Characteristics of Frailty (dependent variables)			
Maximum Leg Extensor Strength (Nm)	-0.41	0.12	<0.001
Isometric Grip Strength (kg)	-0.09	0.04	0.03
Lean mass (kg)	-0.11	0.09	<0.001
Fat mass (kg)	-0.03	0.04	0.43
Total Body Bone Mineral Density (g/cm2)	-0.0005	0.005	0.38
Physical Performance Score (points)	-0.001	0.001	0.17
MHAQ(log) (points)	0.01	0.002	<0.001

β denotes linear regression coefficient. For example: Total Testosterone (TT) decreases 0.12 nmol/l per IU/l Luteinizing Hormone (LH). Modified Health Assessment Questionnaire (MHAQ). Bone density is also adjusted for body mass index.

Figure 1. Relation between Total Testosterone and Luteinizing Hormone (LH) (logarithmically transformed) of Wild-type LH variant subjects, Heterozygote LH variant subjects and Homozygote LH variant subjects.



Luteinizing Hormone and frailty

The relations between LH and the measures of frailty are shown in Table 3. LH was inversely related to maxLES (Figure 2a). LH was also inversely related to lean mass, independent of maxLES (Figure 2b). LH was not associated with bone mineral density or fat mass in the whole group. A positive relation existed between LH and MHAQ. MHAQ and maxLES were strongly related, but the relation between LH and MHAQ was independent of maxLES. Subjects with LH values in the highest quartile had 10.1 % lower maxLES values and 18.7% higher MHAQ values compared to subjects with LH levels in the lowest quartile (after adjustment for age and BMI).

Because we demonstrated that low T levels were accompanied by reduced muscle strength, a multiple regression analysis was performed including LH and T. Both T (total and non-SHBG bound) and LH were significantly, but independently, related to maxLES and isometric grip strength. Multiple regression coefficients of the relations between maxLES and, respectively, LH and non-SHBG-T were $\beta = -0.29 \pm 0.13$ ($P=0.03$) and $\beta = 1.42 \pm 0.56$ ($P=0.01$). Non-SHBG-T and LH were also both related to MHAQ. However, in a multiple regression analysis including LH and non-SHBG-T, only LH remained significantly, inversely related to MHAQ ($\beta = 0.007 \pm 0.002$; $P<0.001$ and $\beta = -0.008 \pm 0.009$; $P=0.40$, respectively).

Luteinizing Hormone variants

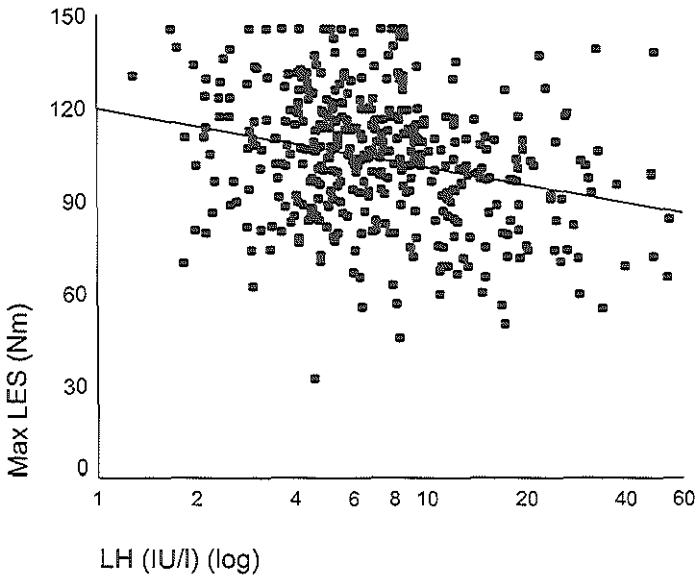
The heterozygote form of the LH variant was present in 12.5% (50 men) in this study population of elderly men. Mean concentrations of TT, non-SHBG-T, LH, SHBG, leptin and albumin were not different in the wild-type LH group compared with the heterozygote LH variant group. The homozygote form of the LH variant was present in only 2 subjects (0.5%). Their TT values were 6.32 nmol/l and 9.97 nmol/l.

The relations between LH and T and age, respectively, were not different in the two LH groups (Table 4).

None of the characteristics of frailty was different in the heterozygote LH variant group compared to the wild-type LH group. Mean values of the measures of frailty of the two variant LH homozygote individuals were also within the ranges of the other groups. The relations between LH on one hand, and the different measures of frailty on the other, in the two LH variant groups are shown in Table 4. Interestingly, LH was positively associated with fat mass and leptin only in the heterozygote LH variant group. This remained after adjustment for T (TT or non-SHBG-T).

Figure 2. Relation between Luteinizing Hormone (LH) in units per litre (IU/l) and A) maximum leg extensor strength (maxLES) in newton meters (Nm) and B) Lean Body Mass in kilograms. Coef denotes coefficient of linear regression.

2 A



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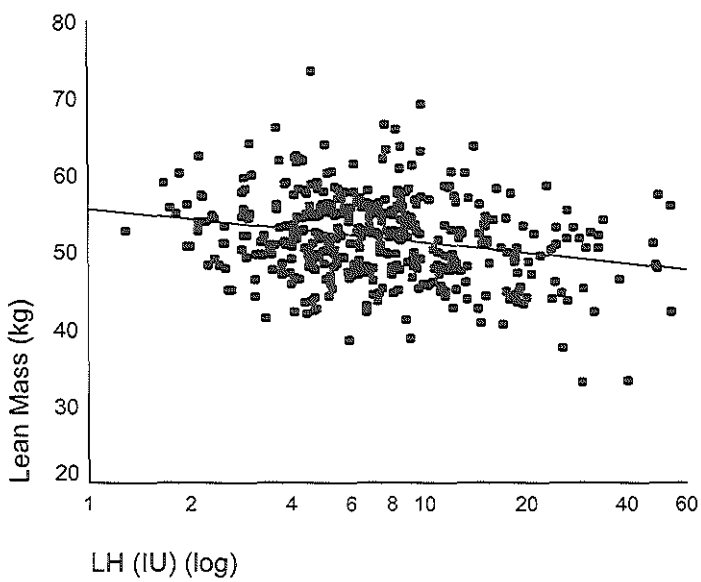

Table 4. Age adjusted linear regression analysis between LH and hormones respectively characteristics of frailty in the wild-type LH group and in the heterozygote LH variant group

	Wild-type LH (n=347)			Heterozygote LH variant (n=50)		
	β ± S.E.		P	β ± S.E.		P
Hormones and SHBG						
Age (yr)	0.44	0.11	<0.001	1.73	0.39	<0.001
Body Mass Index	-0.05	0.02	0.02	0.06	0.04	0.14
Total Testosterone (nmol/l)	-0.11	0.02	<0.001	-0.16	0.04	<0.001
Non-SHBG-Testosterone (nmol/l)	-0.0004	0.0001	<0.001	-0.0005	0.0001	<0.001
SHBG (nmol/l)	0.45	0.10	<0.001	0.05	0.13	0.69
Leptin (log) (μg/l)	-0.004	0.006	0.46	0.03	0.01	0.02
Characteristics of frailty						
MaxLES (Nm)	-0.49	0.14	<0.001	-0.46	0.28	0.11
Isometric Grip Strength (kg)	-0.09	0.05	0.05	-0.15	0.11	0.17
Lean mass (kg)	-0.16	0.04	<0.001	0.022	0.070	0.75
Fat mass (kg)	-0.03	0.04	0.43	0.23	0.07	0.001
TBBMD (g/cm^2)	-0.0001	0.001	0.92	-0.002	0.001	0.19
Physical Performance (points)	-0.001	0.001	0.26	-0.001	0.001	0.26
MHAQ (log) (points)	0.006	0.002	0.007	0.012	0.004	0.004

β denotes linear regression coefficient. Maximum Leg Extensor Strength (MaxLES), total body bone mineral denisty (TBBMD), and Modified Health Assessment Questionnaire (MHAQ).

DISCUSSION

In an independently living population of healthy elderly men, LH significantly increased with age. This gonadotropin was inversely associated with testosterone (TT and non-SHBG-T). LH was, independent of T, negatively related to several characteristics of frailty, *i.e.* maxLES and lean mass and positively with self-reported disability. In this population, 12.5% were heterozygous for the LH variant allele. In the heterozygote LH variant subjects, but not in those with wild-type hormone, LH was positively related to fat mass and leptin.

Conflicting results have been reported concerning the question whether LH increases with age or remains relatively stable (22,23). One reason may be that the aging-induced decrease in testosterone is primarily testicular in some men, mainly due to hypothalamo-pituitary insufficiency in others and of mixed origin in a third group. In our study population LH increased significantly with age. The inverse relation we found between LH and TT, could be due to the rather low T levels of the subjects in our study population. It is likely that in subjects with low T levels, LH cannot increase T, because of a primary disturbance of Leydig cell function.

The strong inverse relation of LH with muscle strength, lean mass and self-reported disability, which is independent of T, suggests that also LH reflects the process of frailty. This association has not been described before. Our observation might imply that LH monitors androgen effects in a different manner than testosterone, probably because LH levels reflect the sum of systemic testosterone levels as well as locally produced 5α-dihydrotestosterone and estrogens which are produced via the local conversion of T. We did measure estradiol in this study; however, this hormone could not explain the independent relation of LH to the characteristics of frailty. The mechanism of this relationship remains to be elucidated.

Previously, a common variant form of LH has been detected in apparently healthy individuals, due to the point-mutation based substitutions described above. There is a large variation in the common frequency of this polymorphism in different populations (0-50%) (15), and presently research is performed to investigate its possible clinical correlates. The prevalence of heterozygozity for the LH variant allele in the current cohort of subjects is in agreement with frequencies measured previously in The Netherlands, which found a mean frequency of 14.3% (95% confidence interval 5.7-22.9) in 63 men and women aged 15 years and older (15). The stability of the prevalence across age groups suggests that there is no selection regarding survival of the LH variant. It has been proposed that the *in vivo* bioactivity of the LH variant is lower than that of the wild-type hormone, due to its shorter circulatory half-time (9,10). On the other hand, this may be compensated for by the higher bio/immuno ratio of the variant hormone at the LH target cell level (8,24). Neither the mean T, SHBG and leptin levels nor the mean values of characteristics of frailty differed between subjects with the wild-type LH and subjects with the heterozygote form of the LH variant. However, from subjective assessment of the findings presented in Figure 1., it appears that the proportion heterozygous for the LH variant was relatively large in a group of subjects with low T and high LH concentrations. This is compatible with a reduced *in vivo* bioactivity of LH in the heterozygous individuals. Further, we observed differences in the relations between LH and leptin as well as fat mass between subjects with the different LH forms. The nature of these differences remains to be explained.

In conclusion, in independently living elderly men, LH increases with age and is inversely related to T. The observed relation between LH and frailty, independent of T, suggests that LH levels reflect the overall serum androgen activity in a different manner from T, possibly reflecting the combined effect of estrogen and androgen feedback action at the hypothalamic-pituitary level. The hormone levels and the degree of frailty are not different in the wild-type and heterozygote LH groups. However, a difference in biological response between the two LH forms, in favor of the wild-type form is suggested, because it seems that a relatively high proportion of subjects heterozygous for the LH variant allele have high LH and low T levels. In addition, a difference exists in the relation between LH and fat mass, respectively leptin, in the heterozygote LH variant subjects versus the wild-type LH subjects.

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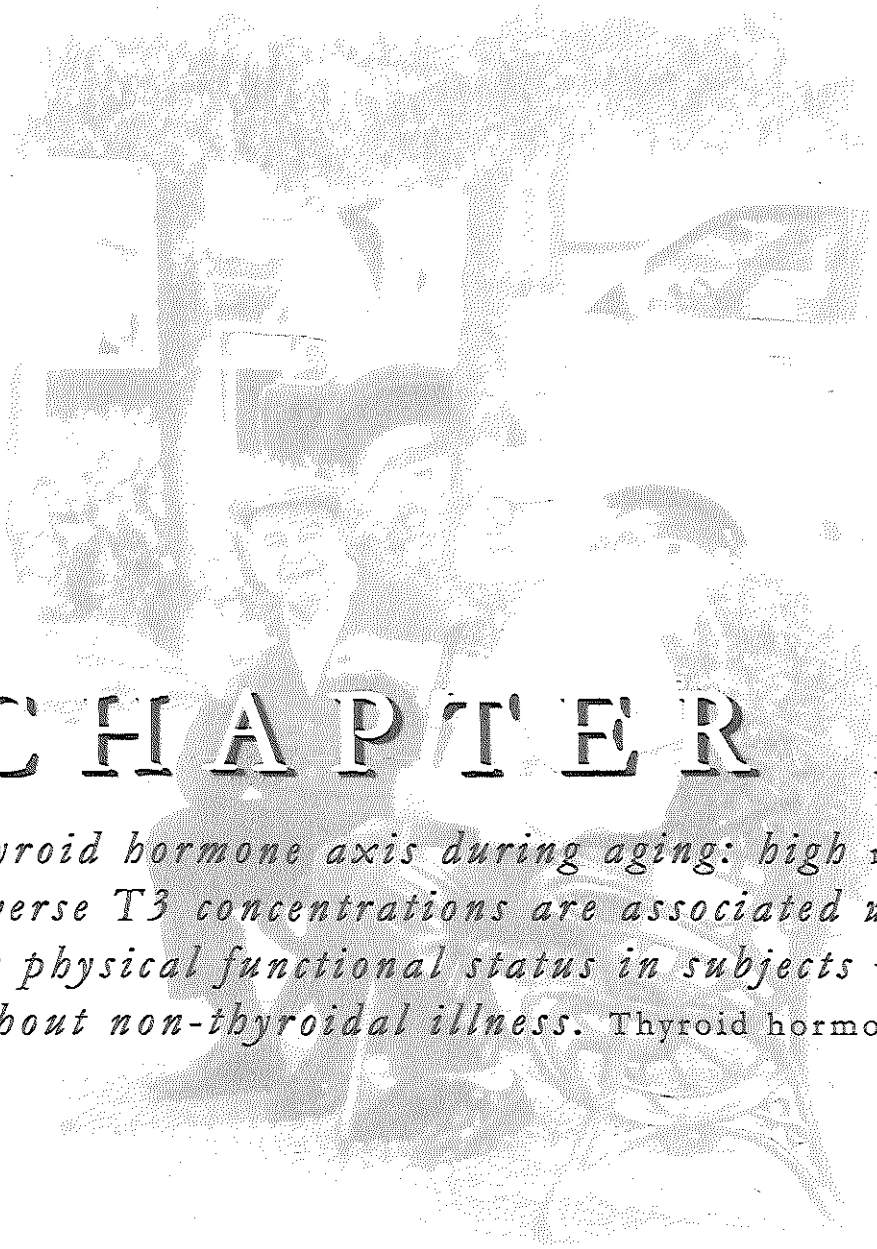


CHAPTER 5

lness. *Thyroid hormone axis during aging: high* reverse T3
high *reverse T3 concentrations are associated with* low
with *low physical functional status in subjects* without n
jects *without non-thyroidal illness.* Thyroid hormone axis d

ABSTRACT

Objective Thyroid hormones regulate the metabolic thermostat by stimulation of the basal metabolic rate. Therefore physiological changes in thyroid hormone concentrations with aging might influence the overall physical functional status. We determined in a cross-sectional setting to what extent thyroid hormone concentrations are related to physical functional status in elderly independently living men.

Methods 403 independently and ambulatory living men (aged 73 to 94 yr) were randomly selected from a population-based sample. Thyroid-stimulating hormone (TSH), free and total thyroxine (FT4 and T4), triiodothyronine (T3), reverse T3 (rT3), thyroxine binding globulin (TBG) and T4 sulfate (T4S) were measured. Physical functional status was estimated by the number of problems in activities of daily living (ADL), a measure of physical performance, leg extensor strength and grip strength, bone mineral density and body composition, including lean mass and fat mass. Quality of life was assessed using a validated questionnaire. Associations were adjusted for age.

Results Serum rT3 concentrations increased significantly with age and with the presence of disease. Thyroid hormone (metabolite) concentrations were related to parameters of physical functional status: independent of disease and after exclusion of subjects with the low T3 syndrome, lower serum total and free T4 and rT3 concentrations and a higher T3/TBG ratio were related with a higher grip strength ($P<0.05$). Also independent of disease, higher free T4, rT3 and T4S levels were associated with a lower physical performance ($P<0.01$). Higher rT3 levels were also associated with more problems in ADL and lower leg extensor strength and bone mineral density and a lower quality of life ($P<0.05$). Some of these relations were partially dependent on the presence of disease

Conclusions In a population of independently living elderly men, serum rT3 concentrations increase with age and with the presence of disease. Higher free T4 and rT3 concentrations are associated with a lower physical functional status. Higher serum rT3 concentrations may result from a decreased peripheral metabolism of thyroid hormones due to the aging process itself and/or to disease, and may reflect a certain catabolic state. Remarkably, rT3 levels remain associated with several parameters of physical function in the absence of systemic disease and the low T3 syndrome. Serum rT3 levels may therefore be a marker of physical function in the elderly, whether or not determined by disease or a poor nutritional status.

INTRODUCTION

Features of aging are in part similar to those of hypothyroidism. In both conditions basal metabolic rate (BMR) decreases (1). Several changes in thyroid hormone concentrations occur during aging: serum thyroid-stimulating hormone (TSH) concentrations decrease in healthy elderly humans, serum total and free triiodothyronine (T3) levels demonstrate a clear, age-dependent decline, whereas serum total and free thyroxine (T4) concentrations remain unchanged (2). These changes are often determined by or occur in parallel with a poor health status (reviewed by Mariotti (2)). Serum reverse T3 (rT3), an inactive metabolite of T4, seems to increase with age (3). Together with the decrease in serum T3 levels, this may indicate a decreased peripheral metabolism of thyroid hormones during aging. Whether T4 sulfate, another inactive metabolite of T4, changes with age is not known. However, evaluation of normal thyroid function in the elderly is complicated by an increased prevalence of non-thyroidal illness and by autoimmune subclinical hypothyroidism (4).

Thyroid hormones are known to regulate the metabolic thermostat by changing the basal metabolic rate. One may hypothesize therefore, that physiological changes in thyroid hormone concentrations might be related with changes in the overall physical functional status in the elderly.

We determined in a cross-sectional setting to what extent thyroid hormone concentrations are related to age as well as to several physical characteristics of aging in independently living, elderly men. In addition, we determined whether potential associations between thyroid hormone concentrations and age and physical status are due to the presence of disease.

METHODS

Subjects.

A cross-sectional, single-centre study was conducted in 403 independently living and ambulatory men, aged 73 years and higher. Names and addresses of all male inhabitants 70 years and older were obtained from the municipal register of Zoetermeer, a medium sized town in the mid-western part of the Netherlands. 1567 men were invited. A total of 886 men did not respond to the mailed invitation in which it was mentioned that only subjects who lived independently and had no severe mobility problems could participate. After exclusion of subjects who did not live independently and subjects who were not physically or mentally able to visit the study centre independently, and subjects with severe systemic illnesses, 403 men participated (25.7%). Participants signed informed consent. The study was approved by the Medical Ethics Committee of Erasmus Medical Center Rotterdam.

We divided the cohort in four groups according to the number of complaints or diseases which were present (0 (n=38), 1 (n=87), 2 (n=71) and ≥3 (n=207)). Present diseases included mainly hypertension, cerebral, coronary and peripheral

atherosclerosis, mild congestive heart failure, chronic obstructive pulmonary disease, diabetes and arthrosis. However, none of the subjects were treated for systemic, infectious, inflammatory or malignant disorders at the time of the investigation.

11 Subjects with known thyroid disease (including subclinical hypothyroidism) or use of amiodarone or corticosteroids were excluded from the analyses. A number of participants were taking medication for hypertension (n=96), angina pectoris or a myocardial infarction more than six months ago (n=85), mild congestive heart failure (n=28), chronic obstructive pulmonary disease (n=40), and diabetes (n=28).

Hormone measurements

Blood samples were collected in the morning after an overnight fast. Serum was separated by centrifugation and stored at -40°C. Thyroid-stimulating hormone (TSH) was measured using an immunometric technique (Amerlite TSH-30, Ortho-Clinical Diagnostics, Amersham, UK). Free thyroxine (FT4), thyroxine (T4), triiodothyronine (T3), and reverse T3 (rT3) were all measured by RIA (Amerlite MAB FT4 assay, Ortho-Clinical Diagnostics, Amersham, UK; (5)). Thyroid binding globulin was also measured by RIA. Intra- and intervariability coefficients of all the assays were below 11%. T4 sulfate (T4S) was measured according to the method of Wu et al. with slight modification (6). It was not possible to measure the T4S concentrations in all samples in a single assay. Intra- and intervariability coefficients of the T4S assay were 17% and 19% respectively in the low concentration range measured in these elderly men.

Physical functional status

Activities of daily living

Self-reported disability or satisfaction in performing activities of daily living (ADL) was assessed by using a self-administered questionnaire modified from the Stanford Health Assessment Questionnaire as described by Pincus et al (7). A high score denotes high impairment in ADL.

Physical performance

Lower extremity function, or physical performance, was assessed as described by Guralnik et al. (8), including measurements of standing balance, walking speed and ability to rise from a chair. The three tests of standing balance were considered in hierarchical difficulty by assigning a single score of 0 to 4 for standing balance. For the 8-foot walk and repeated chair stands, those who could not complete the task were assigned a score of 0. Those completing the task were assigned scores of 1 to 4, corresponding to the quartiles of time needed to complete the task, with the fastest times scored as 4. A summary performance scale (PPS) was created by summing the category scores for the walking, chair stand, and balance test. Mean scores of the three tests as well as of the summary performance scale were comparable to subjects of the same age group investigated by Guralnik (8).

Muscle strength

Isometric grip strength (IGS) was tested using an adjustable hand-held dynamometer (JAMAR dynamometer) in the non-dominant hand (9). Each test was repeated three times and the average, expressed in kilo ponds (kp), was used in the analysis.

Isometric leg extensor strength was measured as described by Hsieh and Philips using the Hoggan MicroFET hand-held dynamometer (10). The measurement requires that the participant, in a seated position, pushes with maximal strength to the dynamometer, which is held at the tibia preferably 33 cm below the knee joint. The investigator holds the dynamometer in the hand with an extended arm and pushes back until the breaking point is reached. To obtain one measure of leg muscle strength, "maximum leg extensor strength" (maxLES) was defined as the maximum strength for the right or left leg in a position of 120°. Statistical analyses were based on the physical unit measurement, moments (N*m), obtained by multiplying the maximum strength (in Newton) and the distance of the dynamometer to the knee joint (in meters).

Bone mineral density (BMD) and Body Composition

Total body bone mineral density (TBBMD) was measured using dual energy X-ray absorptiometry (DXA, Lunar, Madison, WI), as were hip bone mineral densities at the femoral neck, trochanter and Ward's triangle. In addition, total and trunk lean body mass and fat mass were measured (11,12). Quality assurance including calibration was performed routinely every morning for DXA, using the standard provided by the manufacturer.

Height and weight were measured in standing position without shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters.

Well-being

Psychological well-being or quality of life was assessed using a questionnaire with questions pertaining to satisfaction in life according to Henrich and Herschbach (13-15). The Fragen zur Lebenszufriedenheit^{Module} or Questions on Life Satisfaction^{Module} (QLS) has been validated for the Dutch population. The questionnaire is divided in a "general" and a "health" part or module, each including eight items. All items are evaluated on a 5-point scale, ranging from 0 to 4, according to their individual importance (I) and degree of satisfaction (S). As a measure of evaluation, a combination of importance and satisfaction (Ix(2xS-3)) is used. In addition, the sum of the combination values is calculated for each module.

Data analyses

Results are expressed, unless otherwise stated, as mean and standard deviation. Variables which were not normally distributed were logarithmically transformed. Comparisons between groups were made by using Student's *t*-test. Differences are given with corresponding 95% confidence-intervals (CI). Relations between variables were assessed using linear regression stated as linear regression coefficient (β) and 95% Confidence Interval (95%CI). Multiple regression analysis was used to adjust for age and

BMI, and to determine the contribution of different independent variables to the dependent variable. Unless otherwise mentioned all analyses are done after adjustment for age.

RESULTS

Mean age of this population was 77.8 years (range 73 to 94 years). Mean age did not differ within the groups with zero to three or more diseases. Summarized values of the hormone measurements as well as the values in the groups divided according to the presence of disease are presented in Table 1. Serum T3, (free) T4, TSH, TBG, T3/TBG and T4/TBG ratio did not differ between the groups divided according to the presence of disease. Only serum rT3 concentrations differed between the 4 groups, being the highest in the group with 3 or more diseases. 45 subjects had T3 levels below (<1.27 nmol/l) and rT3 levels above (>0.32 nmol/l) the normal range of healthy adults, which is characteristic for the low T3 syndrome. 96 Subjects had T3 concentrations below the normal range of healthy adults, independent of the rT3 concentrations. 158 Subjects had T3 levels within the normal range and rT3 levels above the normal range of healthy adults. Summarized values of the physical characteristics of aging are presented in Table 2.

Relations of the hormones with age

As shown in Table 3, within the population of elderly men, serum rT3 concentrations increased significantly with age, while TSH, free T4 and TBG concentrations did not change with age. There was a tendency in this population for T3 levels to decrease with age. Although T4 levels increased with age in this population (Table 3), none of the subjects had T4 levels above the range of healthy adults (>138 nmol/l). All the relations described in Table 3 were independent of the presence of disease. Serum T4 and rT3 concentrations were strongly related to one another ($r=0.61$, $P<0.001$).

Relations of physical functional status with age

The relations between the parameters of physical functional status and age are demonstrated in Table 4. Problems in performing activities in daily living increased with age, while physical performance, muscle strength and bone density decreased.

Table 1. Descriptive values of the thyroid hormones in a population of 403 elderly men in the total group as well as divided by the number of diseases.

	All subjects		0 diseases		1 diseases		2 diseases		≥3 diseases	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
TSH (mU/l)	1.15	1.05-1.24	1.46	0.87-2.05	1.18	0.96-1.39	1.10	0.92-1.29	1.09	0.99-1.20
Free T4 (pmol/l)	16.6	16.3-16.9	16.4	15.5-17.3	15.9	15.3-16.5	17.1	16.3-17.9	16.7	16.2-17.1
T4 (nmol/l)	80.4	78.8-82.0	75.6	70.5-80.7	78.7	75.0-82.4	81.2	77.3-85.1	81.7	79.6-83.9
T3 (nmol/l)	1.43	1.41-1.45	1.41	1.33-1.48	1.47	1.42-1.52	1.43	1.38-1.48	1.42	1.38-1.45
rT3 (nmol/l)	0.33	0.32-0.34	0.31	0.29-0.34	0.31	0.29-0.32	0.33	0.31-0.35	0.35*	0.33-0.36
TBG (mg/l)	27.2	26.5-27.9	26.0	23.2-28.8	27.7	25.7-29.7	26.8	25.2-28.3	27.4	26.5-28.3
T4 sulfate (pmol/l)	51.9	50.3-53.6	49.6	43.9-55.3	49.9	46.5-53.3	49.5	46.1-52.8	54.1	51.7-56.5
T4/TBG ratio	3.11	3.01-3.20	3.09	2.86-3.33	2.94	2.79-3.10	3.12	2.96-3.28	3.17	3.01-3.33
T3/TBG ratio	0.055	0.05-0.06	0.057	0.053-0.062	0.057	0.054-0.060	0.054	0.053-0.058	0.055	0.052-0.057

* Differences between groups $P<0.01$

Table 2. Summarized values of the physical characteristics in a population of 403 elderly men

	Means	SD	IQ-range	
Age (yr)	77.8	3.6	75	80
Body Mass Index (kg/m²)	25.5	3.0	23.3	27.3
Physical Performance (points)	8.5	2.4	7	10
Activities of daily living (points)	10.7	4.3	8	12
Maximum Leg Extensor Strength (Nm)	103.2	20.9	89.4	117.1
Isometric Grip Strength (kp)	34.3	6.93	30.0	38.7
Total Body Bone Mineral Density (g/cm²)	1.17	0.10	1.11	1.23
Femoral Neck Bone Mineral Density (g/cm²)	0.88	0.14	0.78	0.97
Femoral Ward Bone Mineral Density (g/cm²)	0.72	0.16	0.60	0.82
Femoral Trochanter Bone Mineral Density (g/cm²)	0.85	0.15	0.76	0.94
Total Fat Mass (kg)	21.2	6.4	17.4	24.5
Total Lean Mass (kg)	51.7	5.6	47.8	55.5

Table 3. Relations of the thyroid hormones with age in a population of 403 elderly men

	All subjects		
	β	S.E.	P-value
TSH (mIU/l)	-0.01	0.01	0.24
FT4 (pmol/l)	0.05	0.04	0.23
T4 (nmol/l)	0.68	0.23	0.003
T3 (nmol/l)	-0.006	0.003	0.07
rT3 (nmol/l)	0.005	0.001	<0.001
TBG (mg/l)	-0.01	0.12	0.93
T4S (pmol/l)	1.57	0.22	<0.001
T4/TBG ratio	0.03	0.01	0.02
T3/TBG ratio	-0.003	0.003	0.33

Relations between the thyroid hormones concentrations and the physical characteristics

Since T4, T3 and rT3 were all related to age, all analyses including these parameters were done after adjustment for age. None of the thyroid hormones were related to body mass index.

An inverse relationship was present between serum total T4 concentrations and isometric grip strength and lean body mass (β= -0.06 kp/(nmol/l) [95%CI: -0.10;-0.02], P=0.06, and β= -0.05 kg/(nmol/l) [95%CI: -0.09;-0.02], P=0.002, respectively).

Increasing serum free T4 concentrations were related to lower physical performance score (PPS) (β= -0.11 point/(nmol/l) [95%CI: -0.18;-0.03], P=0.006) and isometric grip strength (β= -0.24 kp/(nmol/l) [95%CI: -0.53;-0.11], P=0.004).

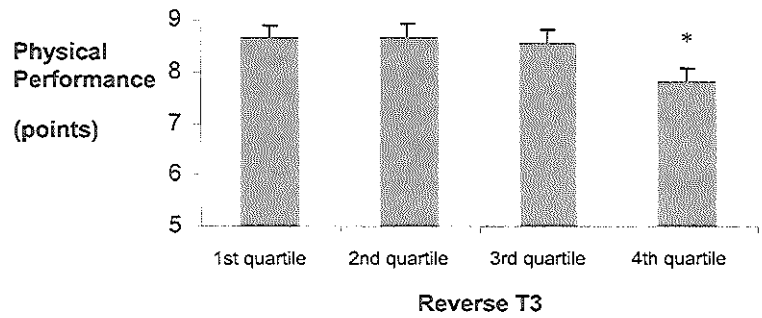
Also serum T3 concentrations were strongly, inversely related to lean mass (β= -3.84 kg/(nmol/l) [95%CI: -5.88;-1.28], P<0.001). The relation between T3 concentrations and lean mass remained significant after adjustment for rT3, TSH, height and muscle strength. Serum T3 levels were, apart from lean mass, not related to any of the other physical characteristics. The T3/TBG ratio was only strongly positively related to isometric grip strength (β=63.2 kp/(nmol/l) [95%CI: 20.9;105.5], P=0.004). This relation was independent of lean body mass.

Higher serum rT3 concentrations were significantly related to lower PPS scores, more problems in activities of daily living, lower muscle strength (leg extensor strength as well as isometric grip strength), lower bone mineral density of the femoral neck and trochanter and lower lean mass. Linear regression coefficients of the relations between serum rT3 levels and the physical characteristics are presented in Table 5A (first column). All analyses including BMD were done after adjustment for age as well as BMI. In Figure 1 it is illustrated that, after adjustment for age, subjects with rT3 concentrations in the highest quartile had significantly lower physical performance scores compared to subjects in the lowest quartile.

Table 4. Associations of physical characteristics with age (years)

	All subjects		
	β	S.E.	P-value
Physical Performance Score (points)	-0.22	0.03	<0.001
Problems in Activities of Daily Living (points)	0.011	0.005	0.02
Isometric Grip Strength (kp)	-0.53	0.09	<0.001
Maximum Leg Eextensor Strength (Nm)	-1.30	0.28	<0.001
Total Body Bone Mineral Density (g/cm2)	-0.003	0.001	0.006
Neck Bone Mineral Density (g/cm2)	-0.002	0.002	0.25
Ward Bone Mineral Density (g/cm2)	-0.003	0.002	0.17
Trochanter Bone Mineral Density (g/cm2)	-0.004	0.002	0.04
Lean mass (kg)	-0.38	0.08	<0.001
Fat mass (kg)	-0.18	0.08	0.02
QLS-General (points)	-0.21	0.37	0.58
QLS-Health (points)	-0.61	0.52	0.24

QLS denotes Quality of Life Satisfaction.



* P=0.01

Figure 1. Quartiles of serum reverse triiodothyronine (rT3) concentrations in relation to physical performance (age adjusted). P denotes significancy towards the first quartile.

Relation between thyroid hormones and physical characteristics and the presence of disease

All the above described relationships between serum rT3 and the physical characteristics were adjusted for the presence of disease in a multiple linear regression model to illustrate the effect of illness on the described relationships. The results of these analyses are shown in Table 5A (second column). In addition, the analyses were repeated after exclusion of the 45 subjects with the low T3 syndrome (T3<1.27 nmol/l and rT3>0.32 nmol/l). These analyses were also performed after adjustment for the presence of disease. The results of these analyses are shown in Table 5B. After exclusion of subjects with low T3 syndrome, the relationship between rT3 levels and leg extensor strength and trochanter BMD were no longer significant. The inverse relations between rT3 concentrations and physical performance, isometric grip strength, and lean mass and the positive relation between rT3 and disability remained significant. After not only exclusion of subjects with low T3 syndrome but also adjustment for the presence of disease, the direction of the linear regression coefficients of the relations between rT3 and the physical characteristics remained the same.

The relationships between total T4 levels and isometric grip strength and lean body mass, as well as the relationship between total T3 and lean body mass and the relation between T3/TBG ratio and isometric grip strength, were all independent of the presence of the low T3 syndrome and the presence of disease.

Also the relationships between free T4 concentrations and physical performance and isometric grip strength remained strongly significant when subjects with the low T3 syndrome were excluded and when was adjusted for the presence of disease (β= -0.11 point/(nmol/l) [95%CI: -0.19;-0.03], P=0.01 and β= -0.30 kp/(nmol/l) [95%CI: -0.53;-0.08], P=0.01, respectively).

Table 5A. Linear regression analyses of associations between rT3 (nmol/l) concentrations and physical characteristics of aging before and after adjustment for the presence of disease

	Before adjustment β (95% CI)	After adjustment β (95% CI)
Physical Performance Score (points)	-3.45 (-6.08; -0.82) [1]	-2.72 (-5.36; -0.09) [2]
ADL (points) *	0.43 (0.07; 0.79) [2]	0.27 (-0.08; 0.63)
Maximum Leg Extensor Strength	-25.3 (-48.2; -2.37) [2]	-20.8 (-43.9; 2.28) [3]
Isometric grip strength (kp)	-8.94 (-16.4; -1.43) [2]	-7.79 (-15.4; -0.21) [2]
Total Body Bone Mineral Density	-0.04 (-0.14; 0.06)	-0.03 (-0.13; 0.07)
Neck Bone Mineral Density (g/cm2)	-0.14 (-0.31; 0.02) [3]	-0.11 (-0.27; 0.06)
Ward Bone Mineral Density (g/cm2)	-0.10 (-0.28; 0.08)	-0.07 (-0.25; 0.12)
Trochanter Bone Mineral Density	-0.18 (-0.34; -0.03) [2]	-0.16 (-0.33; -0.01) [2]
Lean mass (kg)	-7.08 (-13.1; -1.03) [2]	-7.47 (-13.6; -1.34) [2]
Fat mass (kg)	-1.03 (-7.54; 5.49)	-1.70 (-8.31; 4.90)
QLS-General (points)	-7.63 (-38.3; 23.0)	1.71 (-28.9; 32.3)
QLS-Health (points)	-61.2 (-103.6; -18.8) [1]	-38.2 (-79.0; 2.67) [3]

Table 5B. Linear regression analyses of associations between rT3 (nmol/l) concentrations and physical characteristics of aging after exclusion of subjects of subjects with low T3 syndrome, before and after adjustment for the presence of disease

	Before adjustment β (95% CI)	After adjustment β (95% CI)
Physical Performance Score (points)	-4.18 (-7.28; -1.09) [1]	-3.60 (-6.68; -0.51) [2]
ADL (points) *	0.46 (0.05; 0.89) [2]	0.34 (-0.07; 0.76) [3]
Maximum Leg Extensor Strength	-17.1 (-43.9; 9.65)	-12.6 (-39.4; 14.2)
Isometric grip strength (kp)	-9.84 (-18.5; -1.22) [2]	-8.72 (-17.4; -0.05) [2]
Total Body Bone Mineral Density	-0.02 (-0.13; 0.10)	-0.01 (-0.12; 0.11)
Neck Bone Mineral Density (g/cm2)	-0.12 (-0.31; 0.07)	-0.08 (-0.27; 0.11)
Ward Bone Mineral Density (g/cm2)	-0.07 (-0.29; 0.14)	-0.04 (-0.25; 0.18)
Trochanter Bone Mineral Density	-0.15 (-0.33; -0.04)	-0.13 (-0.31; 0.06)
Lean mass (kg)	-7.14 (-14.2; -0.07) [2]	-7.28 (-14.4; -0.15) [2]
Fat mass (kg)	3.38 (-4.18; 10.9)	2.74 (-4.88; 10.4)
QLS-General (points)	-17.0 (-52.5; 18.5)	-8.75 (-44.1; 26.6)
QLS-Health (points)	-50.4 (-99.9; -0.81) [2]	-30.3 (-77.7; 17.1)

For Table 5A and 5B: β denotes linear regression coefficient, 95% CI denotes 95% Confidence Interval. ADL denotes Problems in Activities of Daily Living. * Logarithmically transformed QLS denotes Quality of Life Satisfaction, all analyses are adjusted for age.

[1] P≤0.01 [2] P≤0.05 [3] P≤0.10

Relations between the thyroid hormones and well-being

Serum rT3 and free T4 concentrations were inversely related to the health dimension of the quality of life questionnaire (Table 5A, for free T4; β=-1.79 points/(nmol/l) [95%CI: -2.96;-0.53], P=0.004). Subjects with the highest rT3 concentrations were significantly less satisfied than those subjects with the lowest rT3 levels. The relation between rT3 and health-related quality of life appeared to be partially explained through the presence of disease (Table 5B), while the relation between free T4 levels and quality of life was independent of the presence of disease (after exclusion of the low T3 syndrome and after adjustment for presence of disease β= -1.35 points/(nmol/l) [95%CI: -2.59;-0.12], P=0.03).

T4 sulfate

T4 sulfate (T4S) concentrations increased with age (Table 3). Independent of age, T4S levels were inversely related to PPS (β= -0.02 points/(pmol/l) [95%CI: -0.03;-0.002], P=0.03) and positively to the number of problems in ADL (β= 0.002 points/(pmol/l) [95%CI: 0.0002;0.005], P=0.03). The relation between T4S and PPS remained significant when subjects with low T3 syndrome were excluded and after adjustement for the presence of disease (β= -0.02 points/(pmol/l) [95%CI: -0.04;-0.003], P=0.02), while the relation with problems in activities of daily living was no longer significant.

Serum T4S concentrations were inversely related to general as well as health-related quality of life (β= -0.23 points/(pmol/l) [95%CI: -0.41;-0.05], P=0.01 and β= -0.42 points/(pmol/l) [95%CI: -0.67;-0.17], P=0.001). Subjects with the highest T4S concentrations were significantly less satisfied with life compared to subjects with the lowest T4S concentrations. These relations were independent of the presence of disease.

DISCUSSION

We found in a population of independently living, elderly men, aged 73-94 years, that rT3 and T4S concentrations increased with age. Serum rT3 levels were higher in a subgroup with 3 or more diseases, compared to subjects with less or no diseases. Thyroid hormone (metabolite) concentrations were related to parameters of physical functional status: independent of disease and after exclusion of subjects with the low T3 syndrome, lower serum total and free T4 and rT3 concentrations and a higher T3/TBG ratio were related with a higher grip strength. Also independent of disease, higher free T4, rT3 and T4S levels were associated with a lower physical performance. Higher rT3 levels were also associated with more problems in activities of daily living and lower leg extensor strength and bone mineral density and a lower quality of life. However, these relations were partially or completely dependent on the presence of disease. Serum T4, T3 and rT3 levels were, independent of disease, inversely related to lean body mass. And finally, low free T4 and T4S were related to a better quality of life.

In agreement with previous studies, serum rT3 concentrations increased with age in our population, while there was a tendency for T3 levels to decrease with age (2).

Although there was only a tendency of T3 levels to decrease with age, many subjects had T3 levels below the range of healthy adults. Serum total T4 levels increased with age in our population. This relation could not be explained through an increase in thyroxine binding globulin (TBG) levels. It has to be mentioned, however, that T4 levels were not above the normal range of healthy adults in our population.

These changes in thyroid hormone concentrations may be explained by a decrease in peripheral (hepatic) thyroid hormone metabolism with aging. First, aging may be accompanied by a decreased activity of type I deiodinase (D1) which in turn leads to a decrease in serum T3 due to a reduced peripheral conversion of T4 to T3, an increase in serum rT3 levels due to a reduced rT3 degradation in the liver and increased serum T4S concentrations due to reduced T4S degradation (Figure 2) (16,17). In addition, a reduced selenium intake may contribute to a decreased D1 activity in the elderly, since selenium deficiency is known to reduce the expression of the D1 selenoprotein (18). Second, the observed increase in both rT3 and total T4 levels with aging may in part be explained by a reduced hepatic uptake of rT3 and T4. However, both an impaired D1 activity and a decreased hepatic uptake of thyroid hormones may also be due to disease or a poor nutritional state rather than aging itself. The extent to which the changes in thyroid hormone concentrations and their relations with physical characteristics in this elderly population were due to the aging process per se or to the presence of (non-thyroidal) illness was investigated by examining these relations before and after adjustment for the presence of disease (see below).

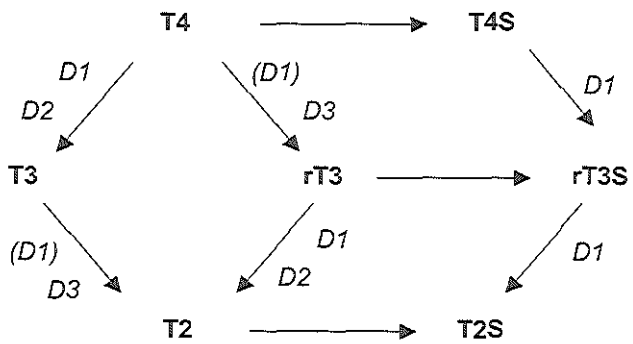


Figure 2. Schematic reproduction of peripheral thyroid metabolism. T4 (thyroxine), T3 (triiodothyronine), rT3 (reverse T3), T2 (diiodothyronine), T4S (T4 sulfate), D1; D2; D3 (respectively type I , II and III deiodinase)

We determined whether changes in serum thyroid hormone concentrations were related to characteristics of the aging process, like physical functional status and quality of life. To our knowledge, these relationships have not been investigated previously. Thyroid hormones are known to play an essential role in many biological processes in nearly every tissue. This is illustrated by the clinical symptoms in hypothyroidism and thyreotoxicosis. We hypothesized therefore that a decreased peripheral thyroid hormone metabolism in the elderly, might lead to worsening of physical functional status. In this study we found that high serum rT3 concentrations were associated with a lower physical functional status, represented by the significant inverse relations between serum rT3 and physical performance score (PPS), muscle strength and bone mineral density (BMD) at the femur as well as by the positive relation of serum rT3 levels and the number of problems in activities of daily living. Higher free T4 concentrations were associated with a lower physical performance and grip strength, while a high T3/TBG ratio, a rough indicator of free T3, was associated with a better grip strength. Further, we observed that low serum rT3 as well as low levels of free T4 were associated with a better health-related quality of life.

As mentioned above non-thyroidal illness is associated with an increase in serum rT3 concentrations (19,20). It needs to be emphasized that the investigated population was relatively healthy and that subjects with systemic infectious or inflammatory and malignant disorders were excluded. Known morbidity included mainly hypertension, atherosclerotic disease, congestive heart failure, chronic obstructive pulmonary disease, diabetes and arthrosis. However, to examine the potential influence of disease on the associations found, the following analyses were made. First, rT3 levels which increased with age were independent of the presence of disease. Second, the decrease in physical functional status with age was not dependent of disease (data not shown). Third, when we adjusted for the presence of disease, similar linear regression coefficients were present in the relations between free T4 and rT3 and the physical characteristics. This despite that rT3 levels were slightly higher in subjects with the presence of several diseases or complaints. Fourth, after exclusion of subjects who met the criteria for the low T3 syndrome, i.e. low T3 and elevated rT3 levels, most relations remained the same. Only the associations between rT3 and leg extensor strength and bone density became non-significant. Finally, we adjusted these associations (after exclusion of the low T3 syndrome) for the presence of disease. Again, the linear regression coefficients remained nearly the same or only slightly decreased for most associations. This may indicate that in the absence of the low T3 syndrome rT3 levels may reflect an individual's physical functional status, partially independent of disease. Increasing rT3 levels could then represent a certain catabolic state, eventually preceding an overt low T3 syndrome. In this respect one's nutritional status may also be a determinant of rT3 concentrations as caloric deprivation is also accompanied by an increase in rT3 levels (3).

Serum T3 and T4 concentrations were significantly and negatively related to lean mass. Both serum T3 and lean mass were normally distributed, and the relationship was therefore not dependent on outliers. This was a very strong association, which as far as we could determine, was not dependent on other characteristics, like height or muscle

strength. It is possible that a different relation would have been found if free T3 instead of total T3 levels were measured. This is suggested by the finding that the T3/TBG ratio was not related to lean mass. The bioactive fraction of total T3 is strongly influenced by the binding of T3 to TBG. Therefore it has to be questioned whether total T3 measurements are biologically relevant and whether the relations we observed between T3 and the physical characteristics are representative for bioactive T3.

Sulfation facilitates the inner ring deiodination of T4 and T3 by D1, whereas the outer ring deiodination of T4S is inhibited, suggesting that sulfate conjugation is a primary step leading to the irreversible inactivation of thyroid hormone. Like rT3, T4S concentrations may increase during disease due to a decreased activity of D1 in the liver. Although borderline of significant, T4S concentrations indeed tended to be higher in subjects with more diseases. Assuming that sulfotransferase expression is relatively constant, we hypothesized that T4S levels might reflect a decreased peripheral thyroid hormone conversion, probably indicating a catabolic state, similar to rT3 levels. We did find a few relations between serum T4S concentrations and parameters of physical functional ability mostly independent of the presence of disease. However, in our population of elderly men, the T4S concentrations measured appeared to be very low, and subject to experimental variation in this low detection range.

In conclusion, in a population of independently living elderly men, serum reverse T3 concentrations increase with age and with the presence of disease. Higher free T4 and rT3 concentrations are associated with a lower physical functional status. Higher serum rT3 concentrations may result from a decreased peripheral metabolism of thyroid hormones due to the aging process itself and/or to disease, and may reflect a catabolic state. Remarkably, rT3 levels remain associated with several parameters of physical function in the absence of systemic disease and the low T3 syndrome. Serum rT3 levels may therefore be a marker of physical function in the elderly, whether or not determined by disease or a poor nutritional status.

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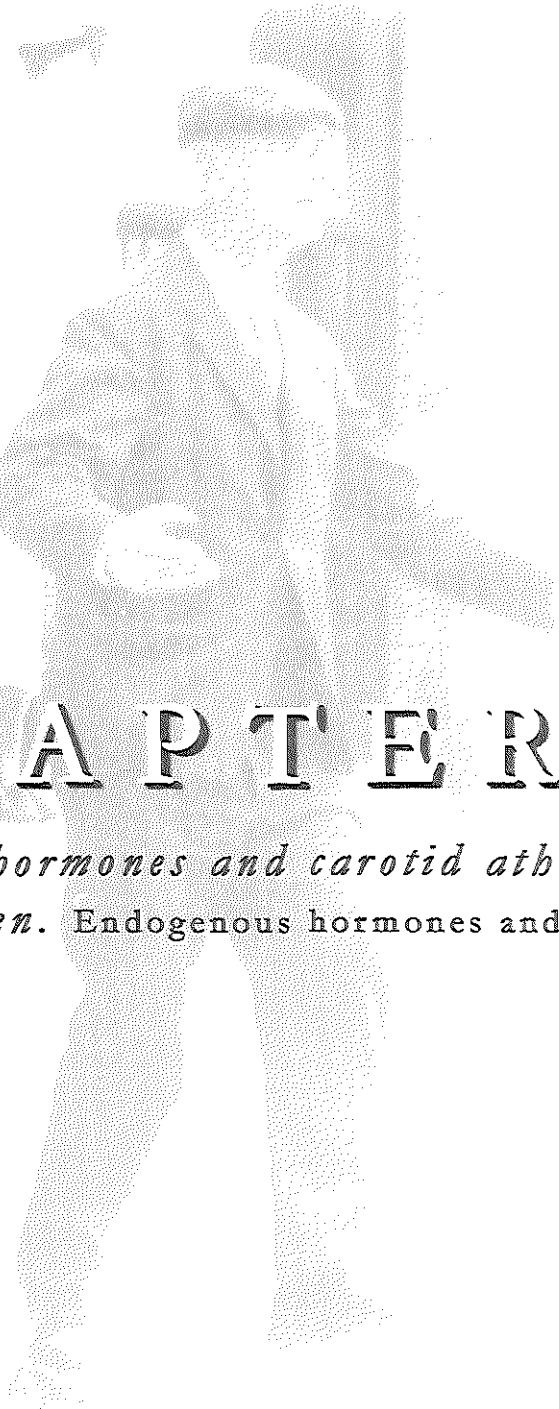


CHAPTER 6

ABSTRACT

Objective The aging process is characterized by a number of gradual changes in circulating hormone concentrations, as well as by a gradual increase in the degree of atherosclerosis. We studied whether serum hormone levels are related to atherosclerosis of the carotid artery in independently living, elderly men.

Methods 403 men (aged 73-94 years) were randomly selected from the general population. Carotid artery intima-media thickness (IMT) was determined. Serum concentrations of testosterone (T), estrone (E1), estradiol (E2), dehydroepiandrosterone (DHEA) and its sulfate, insulin-like growth factor-1 (total and free IGF-I), and its binding proteins-1, -2, and –3 and leptin concentrations were measured.

Results Serum T, E1 and free IGF-1 were inversely related to IMT, after adjustment for age. The strength of these relations was as powerful in subjects with and without prevalent cardiovascular disease. Serum E2, DHEA(S), total IGF-I, IGFBP-1, -2, -3 and leptin showed no association.

Conclusions These findings suggest that endogenous testosterone, estrone and free IGF-I levels may play a protective role in the development of atherosclerosis in aging men.

INTRODUCTION

Cardiovascular disease is the prime cause of death among the elderly in industrialized countries, and a major determinant of chronic disability (1). The burden of atherosclerosis particularly afflicts the increasing older part of the population. Most hormone levels decrease with age, coincident with the age-associated increase in atherosclerotic disease. Consequently, a role in the pathogenesis of cardiovascular disease has been suggested for several hormonal systems.

In men, a beneficial effect of testosterone and of dehydroepiandrosterone sulfate (DHEAS) on cardiovascular risk factors has been described (2-6). Further, in women there is substantial evidence for a protective role of endogenous sex hormones and estrogen therapy in the development of atherosclerosis (7-13).

Also recently, data have been reported to suggest that the insulin-like growth factor (IGF)/ IGF-binding protein system is related to cardiovascular risk factors and the degree of atherosclerosis in an elderly population (14,15).

Most of these studies, however, were performed in relatively young subjects, and have concentrated on the relation between hormones and cardiovascular risk factors. We studied whether serum hormone levels are related to atherosclerosis, as measured by the intima-media thickness of the carotid artery in a population of independently living, elderly men.

METHODS

Subjects

A group of 403 independently living men, aged 73 or above, participated in this study. Participants were recruited by letters of invitation, which were sent to the oldest male inhabitants of Zoetermeer, a medium sized town in the mid-western part of the Netherlands. All participants provided informed consent, and the study was approved by the Medical Ethics Committee of the University Hospital Rotterdam. Participants were judged sufficiently healthy to participate in the study when they were physically and mentally able to visit the study center independently. No additional health related eligibility criteria were used. A number of participants were taking medications for chronic illnesses, including hypertension (n=98), angina pectoris (NYHA class 1 to 3) or a recent myocardial infarction (n=85), mild congestive heart failure (n=28), chronic obstructive pulmonary disease (n=40), and diabetes mellitus (n=33). We divided the cohort by presence (n=139) or absence (n=263) of prevalent cardiovascular disease. Presence of cardiovascular disease was defined as having symptoms of or being treated for angina pectoris, congestive heart failure, claudicatio intermittens, or having a myocardial infarction or cerebro vascular accident in the medical history. Subjects with hypertension were not included in this group, since hypertension is regarded as being a risk factor for cardiovascular disease and not as cardiovascular disease itself. In addition, we divided the cohort by the use (n=123) or none use (n=279) of cardiovascular drug therapies. Cardiovascular drug therapy was defined as using one or more of the following drugs; ACE-inhibitors, calcium antagonists, diuretics, β-

inhibitors (these previous four were not included when they were only used as an hypertension lowering drug), glycosides, nitrates, cordaron, cholesterol lowering drugs and anti-coagulates.

70 subjects smoked at the time of investigation, while 281 subjects did not smoke at present time but had smoked previously. 51 subjects had never smoked.

Height and weight were measured in standing position without shoes. Body mass index was calculated as the weight in kilograms divided by the square of the height in meters. The waist circumference was measured at the level of the umbilicus and the hip circumference was measured at the level of the greater trochanter. The average of two readings was used in the analyses. Waist / hip ratio (WHR), which represents a measure of upper body adiposity, was calculated from these two measurements.

Measures of atherosclerosis

To determine carotid artery intima-media thickness as a quantative measure of generalized atherosclerosis (16), ultrasonography of the left and right common carotid artery and the bifurcation was performed with a 7.5-MHz linear array transducer (ATL Ultramark IV, Advanced Technology Laboratories). A careful search was performed for all interfaces of the near and far wall of the distal common carotid artery and the far wall of the carotid bifurcation (17). The actual measurements of the intima-media thickness were performed off line as described previously (18). A composite measure that combined the mean common carotid artery intima-media thickness and the mean carotid bifurcation intima media thickness (Z-score) was obtained by averaging these two measurements after standardization (subtraction of the mean and division by the standard deviation).

The common carotid artery, carotid bifurcation and internal carotid artery were also evaluated for the presence (yes / no) of atherosclerotic lesions on both the near and far wall of the carotid arteries. Plaques were defined as a focal widening relative to adjacent segments, with protrusions into the lumen composed of only calcified deposits or a combination of calcification and non-calcified material (17). The size of the lesions was not quantified. The number of plaques was used as an indicator of the presence of atherosclerosis.

Lipids

Serum total cholesterol, low density lipid cholesterol, high density lipid cholesterol and triglycerides concentrations were all measured by commercially available radioimmunoassay kits.

Hormone measurements

Blood samples were collected in the morning after an overnight fast. Serum concentrations of total testosterone (nmol/l) and sex hormone binding globulin (nmol/l) were measured by radioimmunoassay using commercial kits (Diagnostic System Laboratories, Webster, Texas, USA). The intraassay coefficients of variation (CV) were 8.1% and 3.0% respectively. The interassay CVs were 10.5% and 4.4% respectively. Serum concentrations of estrone (nmol/l), estradiol (pmol/l), dehydroepiandrosterone (DHEA, nmol/l), and DHEA

sulfate (μmol/l) were also measured by radioimmunoassay using commercial kits (Diagnostic Systems Laboratories). The intraassay coefficients of variation (CV) were 5.6%, 5.3%, 3.8%, and 2.1% respectively. The interassay CV were 10.2%, 8.1%, 8.6%, and 5.1% respectively.

Dissociable free insulin-like growth factor (IGF) -I was measured with a commercially available noncompetitive two-site immunoradiometric assay (Diagnostic System Laboratories). Total IGF-I was measured by an IGF binding protein (IGFBP) blocked radioimmunoassay (RIA) as described previously (19) (Medgenix Diagnostics; intra-assay and interassay CV: 6.1% and 9.9%). IGFBP-1, IGFBP-2 and IGFBP-3 were determined by RIA as described previously (19,20) (respectively Diagnostics System Laboratories Inc; intra-assay and interassay CV: 3.4%, 2.9% and 1.9% and 8.1%, 10.3% and 9.2%). Insulin was measured by a commercially available RIA (Farmacia, Threibel, Germany, intra-assay and interassay CV: 8.0% and 13.7%).). Leptin (μg/l) was also measured by radioimmunoassay (Lilly Research Laboratories, Giessen, Germany).

Data analyses

Results are expressed as mean and standard deviation, unless otherwise stated. Relations between variables were assessed using linear regression for continuous variables, and described as the linear regression coefficient (β) and its standard error as well as the 95% confidence interval (CI). Since an association was present between several hormone concentrations and age, multiple regression analysis was used to adjust for age, as well as to assess the independent contribution of different variables to the dependent variable. We used a t-test to compare continuous characteristics. Differences between groups were stated together with the 95% CI.

RESULTS

Mean age of the population was 77.8 years (range 73-94 years). Descriptive values of the measures of atherosclerosis as well as general characteristics are presented in Table 1. Descriptive values of the hormone levels are presented in Table 2. Mean intima-media thickness (IMT) of the carotid bifurcation (β= 0.05 ± 0.01 mm/year, P=0.001) and the combined IMT of carotid artery (β= 0.05 ± 0.02 mm/year, P=0.01) were both directly associated with age. In our population, serum total testosterone and insulin were not significantly associated with age. Serum estradiol, estrone, DHEA and DHEAS were significantly inversely related with age (respectively β= -1.99 ± 0.80, (pmol/l)/yr, β= -4.90 ± 0.50 (pmol/l)/yr, log transformed β= -0.02 ± 0.006 (nmol/l)/yr and β= -0.04 ± 0.01 (μmol/l)/yr, all P<0.01). Also serum IGF-I, IGFBP-3 and leptin concentrations were inversely related with age (respectively β= -0.85 ± 0.41 (nmol/l)/yr, P=0.04; β= -0.07 ± 0.01 (μmol/l)/yr, P<0.001 and β= -0.02 ± 0.01 (nmol/l)/yr, P=0.04). IGFBP-1 and IGFBP-2 levels were positively related with age (respectively log transformed β= 0.03 ± 0.006 (nmol/l)/yr and β= 0.03 ± 0.004 (μmol/l)/yr, both P<0.001).

Table 1. Baseline characteristics of 403 ambulatory elderly Dutch men

Variable	Obs. (n)	Mean	S.D.
Atherosclerosis			
Mean Intima-Media Thickness of the carotid artery (mm)			
Common Carotid (CCA)	397	0.97	0.15
Bifurcation (BIF)	297	1.33	0.56
Risk Factors			
Cholesterol (nmol/l)			
Total cholesterol	403	5.75	1.14
LDL-cholesterol	403	3.77	1.02
HDL-cholesterol	403	1.34	0.39
Triglycerides	403	1.39	0.84
General characteristics			
Age (years)	403	77.8	3.58
Weight (kg)	403	75.9	10.5
Body Mass Index (kg/cm^2)	403	25.4	3.04
Waist-to-Hip Ratio	400	0.98	0.05
Medical History			
Variable; Presence	Obs (n)	Yes	No
Angina Pectoris	401	60	341
Congestive Heart Failure	402	28	374
Myocardial Infarction	402	66	336
Cerebro Vascular Accident	402	37	365
Claudication Intermittens	402	22	380
Cardiovascular Disease	*402*	*139*	*263*
Hypertension	401	98	303
Diabetes Mellitus	402	33	369
Cardiovascular Medication	*402*	*123*	*279*
Smoking	Never 51	Ever 81	Now 70

Atherosclerosis, gonadal and adrenal hormones

Serum estradiol, dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) concentrations were not related to the combined IMT of the carotid artery, nor after adjustment for age and body mass index (BMI).

Serum testosterone concentrations, however, were inversely related to the mean IMT of the bifurcation and the combined IMT after adjustment for age (P=0.02, Table 3). Serum total testosterone concentrations were inversely related to BMI (β= -0.17 ± 0.05 (nmol/l)/(kg/m^2), P<0.001). Testosterone levels did not differ between subjects with or without hypertension or diabetes. Mean serum testosterone concentrations were significantly higher amongst subjects who had never smoked compared to subjects who had

(difference=1.01 nmol/l, 95%CI; 0.10 – 1.91 nmol/l). The associations between serum testosterone and IMT were independent of the BMI, WHR, the presence of hypertension and diabetes, smoking and serum cholesterol levels (total-, LDL-, HDL-cholesterol, and triglycerides).

Serum estrone concentrations were also inversely related to the mean IMT of the common carotid artery and to the combined IMT, also after adjustment for age (P=0.02, Table 3, Figure 1). Serum estrone levels were positively related to waist/hip ratio (WHR) (β= 0.13 ± 0.07 (nmol/l)/1, P=0.04). Further, subjects with hypertension had significantly higher estrone concentrations compared to subjects without hypertension (difference= 0.01 nmol/l, 95%CI; 0.003 – 0.021 nmol/l). The distribution of estrone concentrations did not differ significantly amongst diabetes and smoking. The association between IMT and estrone concentrations was independent of the BMI, WHR, the presence of hypertension and diabetes, smoking and serum cholesterol levels.

Serum estrone and testosterone concentrations were significantly positively related (r=0.28, P<0.001). We therefore conducted a multiple regression analysis including both estrone and testosterone as the independent variables and IMT of carotid artery as dependent variable. The relation with both estrone and testosterone attenuated and became statistically non-significant (respectively; β=-2.31 ± 1.39 nmol/mm, P=0.10 and β=-0.03 ± 0.02 nmol/mm, P=0.09).

No significant associations were present between serum testosterone, estrone, estradiol, DHEA or DHEAS concentrations and the number of plaques present in the carotid artery.

Table 2. Serum hormone concentrations of 403 ambulatory elderly Dutch men

	Mean	SD
Testosterone (nmol/l)	8.83	2.98
Estrone (nmol/l)	0.102	0.039
Estradiol (nmol/l)	0.098	0.058
Dehydroepiandrosterone (nmol/l)	7.34	3.78
Dehydroepiandrosterone Sulfate (μmol/l)	1.97	1.38
Total Insulin-like Growth Factor-I (μg/l)	100.9	29.2
Free Insulin-like Growth Factor-I (nmol/l)	0.09	0.05
Insulin-like Growth Factor Binding Protein-1 (μg/l)	31.7	15.5
Insulin-like Growth Factor Binding Protein-2 (mg/l)	0.62	0.32
Insulin-like Growth Factor Binding Protein-3 (mg/l)	2.59	0.70
Leptin (μg/l)	5.35	4.19

Atherosclerosis, somatotropic hormones, insulin and leptin concentrations

Serum total insulin-like growth factor (IGF)-I, IGF binding protein (IGFBP)-1, IGFBP-2, IGFBP-3, insulin and leptin concentrations were not significantly related to the IMT of the carotid artery, nor after adjustment for age and BMI.

Serum free IGF-I was inversely related to the mean IMT of the carotid bifurcation after adjustment for age (P=0.05, Table 3, Figure 2). Serum free IGF-I concentrations were not related to BMI or WHR. The distribution of free IGF-I levels did not differ between subjects with or without hypertension, diabetes or smoking. The relation between serum free IGF-I and IMT was independent of the BMI, WHR, the presence of hypertension and diabetes, smoking and serum cholesterol levels.

No associations were present between IGF-I (free nor total), IGFBP-1, IGFBP-2, IGFBP-3, insulin and leptin concentrations and the number of plaques present in the carotid artery.

Table 3. Regression coefficients for intima media thickness of the carotid artery (Z-score CCA + BIF) as the dependent variable and endogenous testosterone, estrone and free IGF-1 levels as the independent variables in 297 elderly men with and without cardiovascular disease

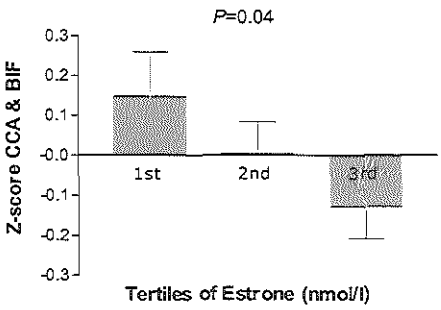
	Z-score CCA + BIF (mm)		
Complete group (n=297)	β	95% CI	
Testosterone (nmol/l)	-0.041	-0.076	-0.006
Estrone (nmol/l)	-3.07	-5.70	-0.44
Free Insulin-like Growth Factor-I (nmol/l)	-1.82	-3.62	-0.013
Free of cardiovascular disease (n=197)			
Testosterone (nmol/l)	-0.034	-0.077	0.009
Estrone (nmol/l)	-3.26	-6.55	0.02
Free Insulin-like Growth Factor-I (nmol/l)	-2.12	-4.30	0.06
With cardiovascular disease (n=100)			
Testosterone (nmol/l)	-0.045	-0.106	0.015
Estrone (nmol/l)	-2.15	-6.52	2.22
Free Insulin-like Growth Factor-I (nmol/l)	-1.33	-4.55	1.90

Common carotid artery (CCA), Bifurcation (BIF), β=coefficient of linear regression: an increase of the independent variable by 1 unit is associated with an increase of intima-media thickness by a factor e^{β}

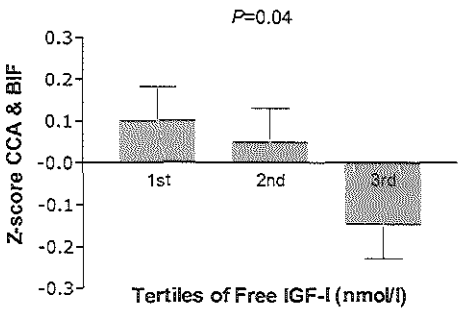
Intima-media thickness, serum hormone levels and prevalent cardiovascular disease

We hypothesized that the above described significant relations between serum estrone, testosterone and free IGF-I concentrations and IMT of the carotid artery are due to cardiovascular disease (CVD) rather than the cause of disease. Therefore we reassessed linear regression between serum hormones and IMT in two groups, namely with and without prevalent cardiovascular disease. The results of these analyses are shown in Table 3. We also performed the same analyses in the two groups with and without cardiovascular drug therapy. Similar results were found as described in Table 3. In addition, serum testosterone, estrone and free IGF-I were not differently distributed amongst subjects with or without CVD or cardiovascular drug therapy.

Figure 1. Mean intima media thickness (IMT) in mm of the combined score of the common carotid artery and the bifurcation in tertiles of **A)** serum estrone and **B)** free insulin-like growth factor–1 (IGF-I) concentrations. *p* denotes the significancy of the third towards the first tertile.



1A



1B

DISCUSSION

In this study among 403 independently living, elderly men, several associations between circulating hormone levels and ultrasonographic measures of atherosclerosis were observed. An increased wall thickness of the carotid artery was related to lower serum testosterone, estrone and free IGF-I concentrations in subjects with prevalent cardiovascular disease as well as in subjects free of cardiovascular disease.

Thickening of the intima-media of the carotid artery is generally considered to be an early marker of generalized atherosclerosis and has been associated with an unfavorable cardiovascular risk profile, other localizations of atherosclerosis (9) and an increased risk of myocardial infarction and stroke (16,21,22). The combined carotid artery intima-media thickness (IMT) measure provides a more precise IMT measurement compared to only common carotid IMT or the carotid bifurcation IMT (21).

Although it is known that testosterone levels influence lipoprotein patterns (3,23), only few studies have directly examined the relation between testosterone and markers of atherosclerotic cardiovascular disease. A study performed by Philips et al. in 55 men (aged 39 to 89) found an inverse relation between serum testosterone and the degree of coronary artery disease (2). We observed that high testosterone concentrations are associated with a reduced intima-media thickness of the carotid artery. It is not possible to discriminate between cause and effect in this cross-sectional study. Low testosterone levels might lead to

an increase in intima-media thickness. On the other hand, atherosclerosis could be widespread and have caused a decrease in testicular blood flow and function. Alternatively, the blood flow to the pituitary could be impaired, leading to low luteinizing hormone concentrations and a degree of secondary hypogonadism, i.e. the hormone changes were the result not the cause of the findings. In addition, high estrone concentrations are associated with reduced intima-media thickness in our population of elderly men. The reason that both estrone and testosterone lost their significant relationship with the intima-media thickness, when adjusted for one another, might be due to the fact that their serum levels were strongly linked. However, the major source for testosterone is from direct testicular secretion.

Few studies have examined the relation between estrogens and atherosclerosis in men (24). In women, estrogen replacement therapy is associated with plaque regression in the carotid artery (10), as well as a delay in thickening of the intima layer of the carotid artery (11). Also in men estrogens may offer some degree of protection against cardiovascular disease by influencing the lipid profile (25,26). We could not demonstrate an association between endogenous serum estradiol concentrations and measures of atherosclerosis. However, it might be that estradiol has a local effect which can not be shown in relations between serum estradiol and measures of cardiovascular disease, since estradiol derives from the conversion of testosterone and estrone, which were both related to measures of cardiovascular disease. In addition, estradiol might change insulin sensitivity of peripheral tissues.

DHEAS has been proposed to be cardioprotective in men (5,6,27). However, its role in the etiology and prevention of coronary disease has been challenged (28), as results from other studies did not support the cardioprotective hypothesis (29). In agreement with Baulieu et al. we did not find supporting evidence for the potential cardioprotective role of DHEA(S), since DHEA(S) concentrations were not associated with the intima-media thickness or the number of plaques in the carotid artery in our population (30).

Recently, data have been reported to suggest that the insulin-like growth factor (IGF) / IGF-binding protein system is related to atherosclerosis in the elderly population (14,31). In several studies it has been demonstrated that growth hormone (GH) deficient adults have an increased intima-media thickness (32), and a high prevalence of hypertension (33). High fasting serum free IGF-I levels have been associated with a reduced number of atherosclerotic plaques, symptomatic cardiovascular disease and lower serum triglycerides levels (31). In the present study, free IGF-I, rather than total IGF-I, was independently related to the mean intima-media thickness of the carotid bifurcation, suggesting that it is the easily dissociable IGF-I fraction which may be able to act on the vascular wall. In diabetic and non-diabetic populations, higher IGFBP-1 levels have been associated with an advantageous cardiovascular risk profile (31,34). IGFBP-1 and -2 are capable of both inhibition and augmentation of IGF-I bioactivity (35). No significant relationships were observed between IGFBP-1 and -2 and atherosclerosis in our elderly population.

It is well established that leptin is positively associated with fat mass, insulin resistance and insulin levels (36). Whether leptin itself, apart from fat mass and insulin levels, is a cardiovascular risk factor is subject to debate (37). Leptin levels in our population were not

related to measures of atherosclerosis.

As already has been touched lightly on, an important question is whether the elevated levels of testosterone, estrone and free IGF-I that are inversely related to carotid intima-media wall thickness are cause or effect. In an attempt to answer this question, we subdivided the cohort by presence or absence of prevalent cardiovascular disease. Although power to infer conclusions is limited, it is shown that the associations between IMT and respectively testosterone, estrone and free IGF-I in subjects free of symptomatic cardiovascular disease were as powerful as in subjects with prevalent cardiovascular disease. This is illustrated by the same direction of the linear regression coefficient as well as by the fact that there was no significant difference between these associations. Similar results were found for subjects with and without cardiovascular drug therapy. These results suggest that the findings are not due to cardiovascular disease, but give a hint that low hormone levels are indeed cause of disease. Of course follow-up studies and preferably intervention studies should be performed to confirm these preliminary conclusions.

In summary, testosterone, estrone, and free IGF-I concentrations, appear to be linearly, inversely related to atherosclerosis as measured by the intima-media thickness of the carotid artery in elderly men. The fact that the associations were as strong in the group free of cardiovascular disease as in the group with prevalent disease, suggest that testosterone, estrone and free IGF-I levels may play a protective role in the development of atherosclerosis in aging men.

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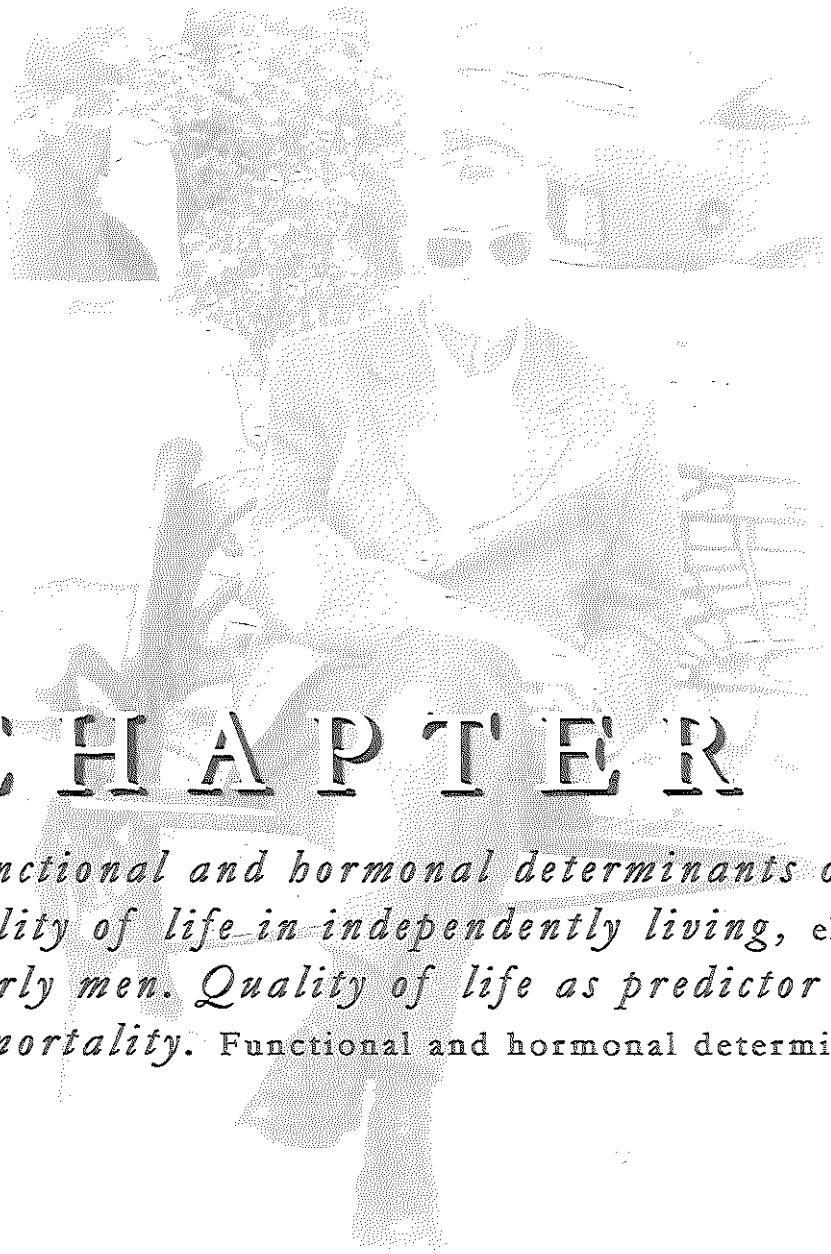

CHAPTER 7

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ABSTRACT

Objective To assess which health, physical and hormonal factors are related to quality of life, and to assess whether quality of life is predictive of mortality in a population of independently living elderly men.

Methods 403 men (aged 73-94 years) were randomly selected from the general population. Four years after the initial investigation, data of death were recorded. At baseline, Quality of Life (QoL) was assessed using a validated questionnaire, which consists of three modules directed at general factors (QLS-General), health factors (QLS-Health), and factors related to hormone deficiency (QLS-Hormone). Physical characteristics of aging included problems in activities of daily living, a measure of physical performance, muscle strength, bone mineral density and body composition. Testosterone, estrone, estradiol, dehydroepiandrosterone (DHEA), DHEAS, sex hormone binding globulin (SHBG), insulin-like growth factor-I (IGF-I), IGFBP-1, IGFBP-2, IGFBP-3 and insulin were all determined by RIA.

Results QoL did not change with age. QLS-Health was closely related to prevalent morbidity at baseline, like prostate disease and congestive heart failure. Muscle strength, physical performance and subjective functional ability were strongly positively related to QLS-Health. Serum bioavailable testosterone weakly correlated to QLS-Health, while DHEA(S) did not. Estradiol and IGFBP-3 concentrations were strongly positively associated to all QLS modules, while cortisol and IGFBP-1 were inversely related with QLS-General and QLS-Hormone. IGFBP-1 was also inversely related to QLS-Health. Serum IGF-I concentrations were weakly positively associated with QLS-Hormone only. QLS-Health and QLS-Hormone were both predictive of mortality (OR=0.99 ± 0.003, P=0.003 and OR=0.99 ± 0.004, P=0.05). In addition, subjects with scores in the lowest quartiles of the questionnaire (the least satisfied) died significantly earlier compared to the subjects with scores in the highest quartiles (P=0.001 and P=0.01 respectively).

Conclusions Irrespective of the health status, physical and psychological well-being are strongly related in independently living, elderly men. In this population, estradiol and IGFBP-3 levels are powerful direct predictors of QoL. Cortisol and IGFBP-1 levels, however, are inverse predictors. These data, although collected in a cross-sectional study indicate that exercise in order to improve muscle strength and/or functional ability, but perhaps also hormonal intervention, which aims at increasing estradiol bio-availability and/or IGFBP-3 levels might further improve quality of life in elderly men. The importance of defining determinants of quality of life is shown by the fact that quality of life itself is an important predictor of mortality.

INTRODUCTION

Psychological well-being and quality of life are important determinants of successful aging (1). Health and socioeconomic factors in association with psychological well-being have been studied extensively (2-5). Modest reductions in the capacity to perform common physical functions may already prevent full participation in productive and recreational activities of daily life. Although a strong positive relation is expected between physical and psychological well-being, this relation has hardly been directly examined in healthy elderly subjects. Furthermore hormonal activity might be related to quality of life. Some studies have reported improvement in the sense of well-being during testosterone supplementation in elderly men (6-8). In addition, several studies have reported that estrogen replacement therapy of post-menopausal women improves quality of life (9-12). Controversy exists concerning the potential beneficial effects of dehydroepiandrostenedione (DHEA) administration on well-being in elderly men (13-15). Finally, it has been demonstrated that growth hormone treatment of adult patients with growth hormone deficiency increases psychological well-being (16-18).

Some studies have shown that quality of life is a predictor of mortality after coronary artery bypass surgery (19), in patients with congestive heart failure (20) and in patients with metastatic colorectal cancer (21). With regard to quality of life as predictor of mortality in relatively healthy older populations, only one study recently demonstrated that in a large study amongst older Mexican Americans, subjects (men and women) with low scores on an emotional well-being questionnaire had a significant higher risk of mortality compared to subjects with high scores (22).

In the present study we have investigated which health factors, physical and hormonal factors are related to psychological well-being in a population of independently living elderly men. In addition, in this same population, we investigated whether quality of life by itself might be a predictor of mortality.

METHODS

Subjects

A cross-sectional, single-centre study was conducted in 403 independently living men, aged 70 years and older. Names and addresses of all male inhabitants 70 years and older were drawn from the municipal register of Zoetermeer, a medium sized town in the mid-western part of the Netherlands. 1567 men were invited. A total of 886 men did not respond to the mailed invitation in which it was mentioned that only those subjects who lived independently and had no severe mobility problems could participate. After exclusion of subjects who did not live independently and who were mentally not fit to visit the study centre independently and undergo the investigations, eventually 403 men participated (25.7%), aged between 73 and 94 years. The main reason not to participate among the respondents was because they were ill and currently seen by a specialist or general practitioner (28%), while 16% was excluded on the basis of physical (10%) or mental (6%)

problems. Participants signed an informed consent. The study was approved by the Medical Ethics Committee of the Erasmus University Hospital Rotterdam. No additional health-related eligibility criteria were used.

A 21 item medical history was obtained by questionnaire according to following groups: musculoskeletal impairments (including arthrosis and fractures); cardiovascular impairments (including symptoms or treatment of angina pectoris, congestive heart failure, hypertension, arythmias, myocardial infarction, cerebrovascular accidents and shortness of breath); prostate problems (hyperplasia and cancer);other malignancies; endocrine disorders (diabetes mellitus and thyroid disease); and symptoms like dizziness and impaired vision which limits mobility. Further a physical examination was performed. From the results of these questions it appeared that a number of participants were taking medications for chronic illnesses, including hypertension (n=98) and mild congestive heart failure (n=28). However, in retrospect, the use or no use of these medications did not influence the relations described in this study.

Four years after the initial investigation, 75 men had died (8 in the first year, 16 in the second year, 21 in the third year and 30 men in the fourth year after the initial investigation had taken place). For the subjects who had died during the four year follow-up, the number of months between death and the initial investigation was recorded.

Psychological well-being

Psychological well-being or quality of life was assessed using a questionnaire with questions pertaining to satisfaction in life according to Henrich and Herschbach (23-25). The Fragen zur Lebenszufriedenheit^{Module} or Questions on Life Satisfaction^{Module} (QLS) combines three features: economy, modular structure, and individual weighting of items. The questionnaire used consisted of three modules. The three modules of the questionnaire used consist of a single sheet of paper each, containing both instructions and 8 items. They can be completed in a few minutes, and no difficulties in understanding were observed in the group of elderly men studied. The first module addressed 8 general questions ("general"-module), the second module consists of 8 questions regarding general health ("health"-module), and the third module, with 9 questions, was developed on the basis of a number of interviews in adults with hypopituitarism ("hormonal"-module) (Table 1). All items are evaluated on a 5 point scale, ranging from 0 to 4, with the highest score indicating extremely important and respectively very satisfied. Of the 8 items asked first the individual importance is asked and subsequently, the personal degree of satisfaction is being asked of these same 8 items. As a measure of evaluation, a combination of importance (I) and satisfaction (S) (Ix[(Sx2)-3]) is used for each item. In addition, the sum of the combination is calculated for each module.

Physical characteristics of successful aging

Muscle Strength

Isometric leg extensor strength was measured as described previously using the Hoggan MicroFET hand held dynamometer (26,27). The measurement requires that the participant, in a seated position, pushes with maximal strength to the dynamometer, which is held at the tibia. The investigator holds the dynamometer in the hand and pushes back until the

breaking point is reached. The measurements were done three times and the maximum performance at each position was recorded. To obtain one measure of leg muscle strength, 'maximum leg extensor strength' (maxLES) was defined as the maximum strength for the right or left leg in a position of 120°.

Bone mineral density (BMD) and Body Composition

Total body bone mineral density (TBBMD) was measured using dual energy X-ray absorptiometry (Lunar, Madison, WI), as were hip bone mineral densities at the femoral neck, trochanter and Ward's triangle. In addition total and trunk lean body mass and fat mass were measured (28,29). Quality assurance including calibration was performed routinely every morning for DEXA, using the standard provided by the manufacturer.

Height and weight were measured in standing position without shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2).

Physical Performance

Lower extremity function, or physical performance, was assessed as described by Guralnik et al. (30), including measurements of standing balance, walking speed and ability to rise from a chair. A summary performance scale (PPS), ranging from 0 to 12 points, was created by summing the category scores for the walking, chairstand, and balance test.

Activities of daily living (ADL)

Satisfaction in performing activities of daily living (ADL) was assessed by using a self-administered questionnaire modified from the Stanford Health Assessment Questionnaire (MHAQ) as described by Pincus et al (31). All items are evaluated on a 4 point scale. A high score denotes high impairment in ADL.

The Mini-Mental State Examination (MMSE) was used to assess cognitive function (32).

Hormone measurements

Blood samples were collected in the morning after an overnight fast. Serum was separated by centrifugation and stored at -40°C. Serum concentrations of total testosterone (TT, nmol/l), and sex hormone binding globulin (SHBG, nmol/l) were measured by radioimmunoassay using commercial kits (Diagnostic System Laboratories, Sinsheim, Germany). The intra-assay coefficients of variation (CV) were respectively 8.1% and 3.0%. The inter-assay CVs were respectively 10.5% and 4.4%. In addition, serum concentrations of estrone (E1, nmol/l), estradiol (E2, nmol/l), DHEA (nmol/l), and DHEAS (μmol/l) were also measured by radioimmunoassay (RIA) using commercial kits (Diagnostic System Laboratories). The intra-assay coefficients of variation (CV) were respectively 5.6%, 5.3%, 3.8%, and 2.1%. The inter-assay CV were respectively 10.2%, 8.1%, 8.6%, and 5.1%. Non SHBG bound testosterone (nmol/l) and non SHBG bound E2 (nmol/l) were calculated according to a method described by Södergård et al. (33). Cortisol (nmol/l) and insulin (mIU/l) were measured by RIA (Diagnostic System Laboratories). Albumin (g/l) was measured by photometry using a commercial kit (ALB, Boehringer-Mannheim).

Total IGF-I was determined by a IGFBP-blocked RIA in the presence of large excess of IGF-II as described (34). Intra- and interassay CVs were 1.6% and 6.4% respectively. IGFBP-1, IGFBP-2, and IGFBP-3 were all measured with inhouse RIA's as described

previously with intraassay CVs of 3.4%, 2.9% and 1.9% and interassay CVs of 8.1%, 10.3% and 9.2% respectively (34). Insulin was measured by a commercially available radioimmunoassay (Medgenix Diagnostics, intra-assay and inter-assay CV: 8.0% and 13.7%).

Data analyses

Results are expressed, unless otherwise stated, as mean and standard deviation with the interquartile (IQ) range. Variables which were not normally distributed were logarithmically transformed. Comparisons between groups were made by using Students *t*-test. Differences are given with corresponding 95% confidence-intervals (CI). Relations between variables were assessed using linear regression for continuous variables and logistic regression for binary variables, stated as linear regression coefficient (β) or Odds ratio and standard errors. Multiple regression analysis was used to assess the contribution of different independent variables to the dependent variable. Partial correlations between variables were assessed by calculating Pearsons product *r*. Cox regression was done to analyse the relative risk of mortality, stated as B and its standard error. Analyses were performed using Stata statistical package (StatCorp 1997. Stata Statistical Software: Release 5.0).

RESULTS

Summarized values of the quality of life questionnaire (QLS) are described in Table 1. 14 subjects which did not fully complete the questionnaire were excluded from the analyses, since the combination of the modules of QLS-questionnaire is not representative if not all the items have been answered. No differences in health, physical characteristics or serum hormones were found between these 14 excluded and the included subjects. Three of the subjects who did not fill in the questionnaire had died after 4 years, while 11 subjects were still alive. Summarized values of the physical characteristics have been previously described (35). Mean age of this population was 77.8 ± 3.6 years (range 73-94).

None of the summarized scores of the general, the health or the hormonal module were related to age. Only the question about leisure time of the general module (QLS-General) and the questions concerning mobility and vision/hearing of the health module (QLS-Health) were inversely related with age (β= -0.15 ± 0.08 points/year, P=0.05; β= -0.28 ± 0.10 points/year, P=0.005 and β= -0.36 ± 0.10 points/year, P<0.001, respectively).

Relations with medical history

Mean scores especially of QLS-Health, were significantly lower in subjects which answered yes on several questions in the medical history (for example: angina pectoris, dyspnea, subjects with a cerebral vascular accident, dizziness). Subjects with complaints of prostatism had a mean score of the health module which was 14.07 ± 4.03 points lower than subjects which did not have complaints of prostatism (P<0.001). Prostate cancer seemed to interfere most in all three modules of the quality of life questionnaire. In Figure 1 is

illustrated that subjects with 5 or more complaints or diseases (established by the 21 item

Table 1. Summarized values Quality of Life questionnaire (QLS)

QLS-General	Obs. (n)	Mean (points)	± S.D.	Min.	Max.
Friends / acquaintances	389	8.02	5.38	-9	20
Leisure time / hobbies	389	8.21	5.50	-9	20
Health	389	10.35	7.53	-12	20
Income / financial security	389	8.86	5.24	-12	20
Occupation / work	389	5.40	5.78	-12	20
Housing / living conditions	389	10.95	5.07	-9	20
Family life / children	389	12.54	5.77	-6	20
Partner relationship / sexuality	389	9.02	6.96	-12	20
Total score general module	*389*	*67.97*	*26.18*	*-56*	*140*
QLS-Health	Obs. (n)	Mean (points)	± S.D.	Min.	Max.
Physical condition / fitness	388	6.40	6.26	-12	20
Ability to relax / stay on an even	388	7.73	5.59	-12	20
Energy / zest for life	388	9.23	6.14	-12	20
Mobility (e.g. walking, driving)	388	8.06	6.92	-12	20
Vision and hearing	388	7.01	7.52	-12	20
Freedom from anxiety	388	9.02	6.25	-12	20
Freedom from aches and pains	388	8.40	7.09	-12	20
Independence from help / care	388	11.77	6.55	-12	20
Total score health module	*388*	*67.62*	*36.73*	*-54*	*160*
QLS-Hormone	Obs. (n)	Mean (points)	± S.D.	Min.	Max.
Ability to withstand stress	386	5.52	5.06	-9	20
Figure/ appearance	386	5.59	4.46	-9	20
Self-confidence	386	8.77	5.42	-12	20
Ability to become sexually	386	3.62	5.11	-12	20
Ability to concentrate	386	7.26	6.50	-12	20
Physical endurance	386	5.27	6.50	-12	20
Initiative/ activity	386	5.72	5.56	-9	20
Coping with anger	386	5.16	5.84	-9	20
Coping with bustle	386	4.27	6.54	-12	20
Total score hormonal module	*386*	*54.18*	*35.48*	*-50*	*142*

questionnaire) were significant less satisfied with life compared to subjects with zero or one complaint or disease. Interestingly all anamnestic aspects in the medical history which were found to be lowering quality of life turned out to be statistically most significant in the "health"-module, with the "general"- or "hormonal"-module not adding information in any instance.

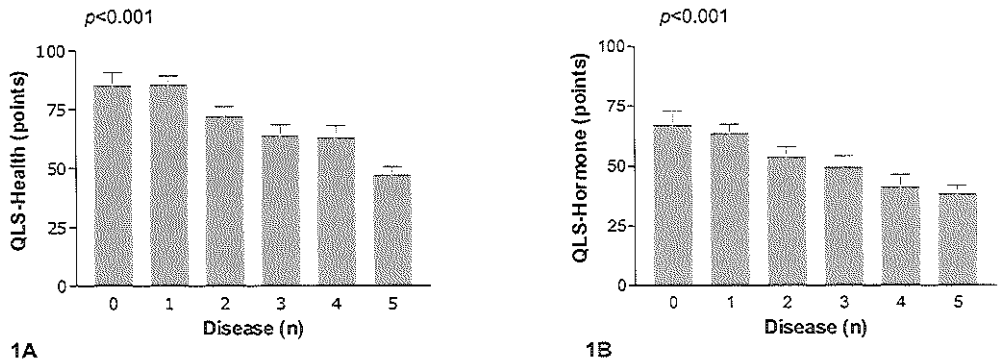


Figure 1. Sores of the quality of life questionnaire according to the number of diseases of **A)** the health module and **B)** the hormonal module. *p* denotes the significancy of 5 towards 0 diseases.

Relations with physical characteristics

These relations are described in Table 2. Since age was not related to QLS, we did not adjust for age. Body Mass Index (BMI) was not related with QLS. Isometric grip strength (IGS) and maximum leg extensor strength (maxLES) were both positively related to all modules of the QLS questionnaire. MaxLES was 19%, 45% and 44% higher, respectively in subjects which scored in the highest compared to the lowest tertile of QLS-General, QLS-Health and QLS-Hormone, respectively (Figure 2).

Bone mineral density (BMD) was positively related to all three modules. BMD and muscle strength were positively related ($r=0.25$, $P<0.001$). Therefore, a multiple regression analysis was performed. After adjustment for maxLES, BMD was no longer significantly related to QLS. Body composition, including lean body mass and fat mass, were not related to QLS.

Physical performance was positively related to QLS-Health and QLS-Hormone. Impairments in activities of daily living (ADL) were inversely related to all three modules. This implies that in our population of elderly men, subjects with more problems in activities of daily living were less satisfied with life. ADL was independently, inversely related to physical performance ($r=-0.54$, $P<0.001$) and maxLES ($r=-0.34$, $P<0.001$). Physical performance and maxLES were also strongly associated ($r=0.40$, $P<0.001$). ADL and maxLES were both independently related with QLS. However, part of the relation between QLS-Health and physical performance was explained through maxLES (linear regression coefficient decreased from 3.46 ± 0.76 to 1.67 ± 0.81), while physical performance was no longer associated with QLS-Hormone after adjustment for maxLES. Interestingly, as was the case for the relationship with the anamnestic variables, the QLS-Health module turned out in all instances to have the closest relation with the physical characteristics measured, without the QLS-General or -Hormone modules adding any information.

To be certain that the association between muscle strength and quality of life was independent of physical complaints and diseases, we adjusted for the number of presence of these diseases (0, 1, 2, 3, 4 or≥ 5).The number of diseases as well as maxLES remained independently related to QLS-Health (respectively β= -7.22 ± 0.98 point/number, P=<0.001 and β= 0.48 ± 0.08 point/Nm, P<0.001).

Table 2. Results from linear regression of Quality of Life on physical characteristics.

	QLS-General (points)			QLS-Health (points)			QLS-Hormone (points)		
	β	S.E.	P	β	S.E.	P	β	S.E.	P
Age (years)	-0.21	0.37	0.58	-0.61	0.52	0.24	-0.62	0.50	0.22
BMI	0.81	0.44	0.06	0.58	0.62	0.34	1.48	0.59	**0.01**
Muscle Strength									
IGS (kg)	0.44	0.19	**0.02**	1.11	0.26	**<0.001**	0.59	0.26	**0.02**
MaxLES	0.31	0.06	**<0.001**	0.59	0.09	**<0.001**	0.44	0.09	**<0.001**
Bone Mineral Denisty (g/cm²)									
TotalBody	31.2	13.4	**0.02**	40.5	19.0	**0.03**	36.6	18.4	**0.05**
Neck	22.9	9.20	**0.01**	37.8	12.9	**0.004**	34.2	12.5	**0.007**
Ward	23.6	8.18	**0.004**	31.5	11.6	**0.007**	28.1	11.2	**0.01**
Trochanter	25.2	9.04	**0.006**	37.2	12.7	**0.004**	28.7	12.4	**0.02**
Body Composition (kg)									
Lean Body	0.36	0.23	0.12	0.49	0.33	0.13	0.45	0.32	0.16
Fat Mass	-0.01	0.23	0.98	-0.03	0.33	0.93	0.23	0.32	0.46
Functional ability (points)									
PPS	0.94	0.54	0.07	3.46	0.76	**<0.001**	1.67	0.75	**0.03**
MHAQ	-1.47	0.30	**<0.001**	-3.45	0.42	**<0.001**	-1.88	0.42	**<0.001**

β= linear regression coefficient. For example: QLS-General increases 0.31 point per Nm Maximum Leg Extensor Strength (MaxLES). IGS=Isometric Grip Strength, PPS=Physical Performance Score, MHAQ= Modified Health Assesment Questionnaire, which reports on the activities of daily life.

Relations with hormones levels

These relations are described in Table 3. Only serum non-SHBG-bound testosterone levels were just related to QLS-Health, but not to QLS-Hormone. Serum estradiol (total and non-SHBG bound E2) was very significantly associated to all three modules tested. Subjects with serum non-SHBG-bound E2 levels in the highest tertile scored 9.9% higher in QLS-General, 20% higher in QLS-Health and 31% higher in QLS-Hormone compared to subjects with serum non-SHBG-bound E2 levels in the lowest tertile (Figure 3a). Serum DHEA levels were not related to quality of life. DHEAS was positively related to QLS-Health only. However, DHEAS and E2 were strongly related and after adjustment for E2, DHEAS was no longer associated with the health module. An inverse association was present between serum cortisol and QLS-General and QLS-Hormone (Figure 4a). Cortisol was not associated with QLS-Health.

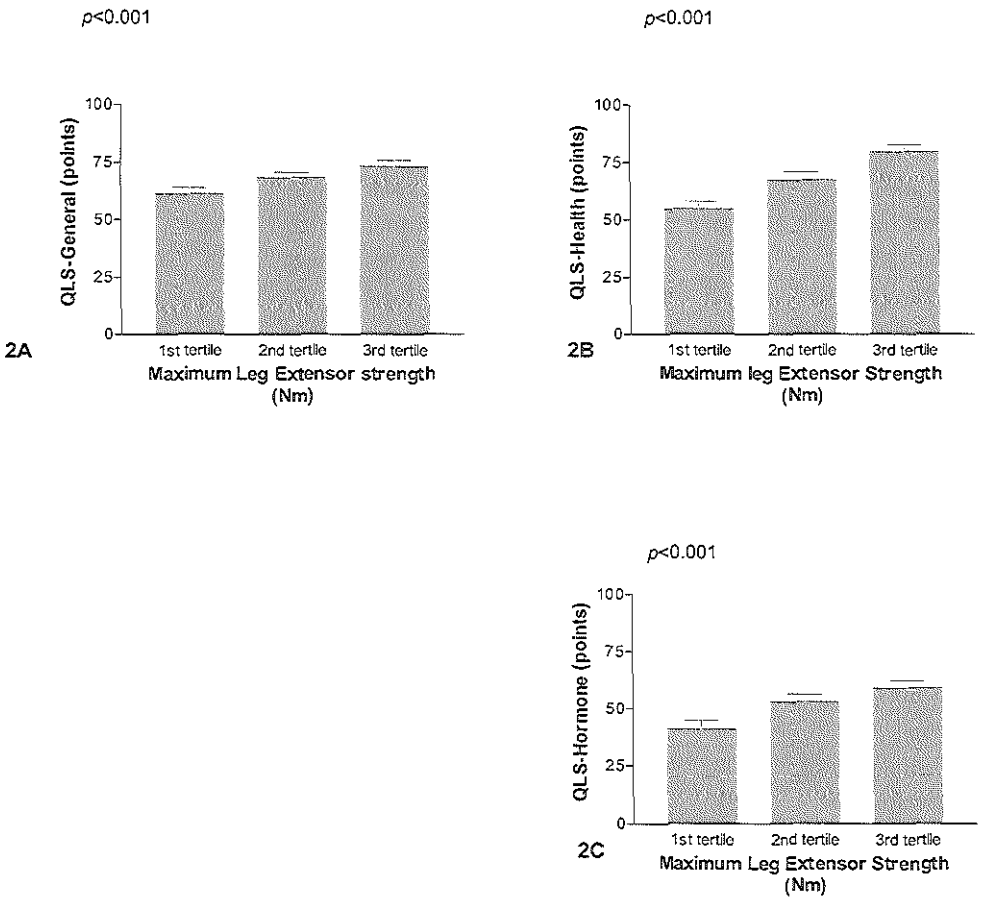


Figure 2. Scores of the quality of life questionnaire in tertiles of maximum leg extensor strength of **A)** the general module, **B)** the health module and **C)** the hormonal module. *p* denotes the significancy of the third towards the first tertile.

Serum IGF-I was not associated with QLS measured in the general and the health module. It was, however, positively related to QLS-Hormone. Its binding proteins were related to all three QLS modules, but most significantly to QLS-Health. Serum IGFBP-3 positively, and IGFBP-1 and -2 inversely. IGFBP-3 concentrations were independent of IGF-I, IGFBP-1, -2 or insulin levels related to QLS (Figure 3b). Also serum IGFBP-1 levels were independent of physical characteristics and insulin levels related to QLS (Figure 4b). The relation between IGFBP-2 and QLS, however, seemed to be dependent on the relation of maxLES and QLS. Serum IGFBP-2 concentrations and muscle strength were strongly inversely related. In view of these findings, a multiple regression analysis was performed including QLS as the dependent variable and maxLES and IGFBP-2 as the independent variables. MaxLES remained significantly related to QLS, while IGFBP-2 did not. Leptin an albumin levels were not associated with quality of life.

Table 3. Results from linear regression analyses of Quality of Life on hormones.

	QLS-General (points)			QLS-Health (points)			QLS-Hormone (points)		
	β	± S.E.	P	β	± S.E.	P	β ± S.E.		P
Androgens									
TT (nmol/l)	0.68	0.44	0.13	0.90	0.62	0.15	0.11	0.60	0.85
FT (pmol//)	38.8	100.6	0.70	-35.6	140.6	0.80	34.9	28.4	0.22
SHBG (nmol/l)	-0.04	0.09	0.65	-0.14	0.13	0.29	-0.21		**0.03**
Non-SHBG-T	1.26	0.70	0.07	1.91	0.98	**0.05**	1.16	0.95	0.23
DHEA (nmol/l)	-0.42	0.35	0.23	0.30	0.49	0.53	-0.37		0.43
DHEAS (mmol/l)	0.40	0.97	0.68	3.22	1.34	**0.02**	1.05	1.31	0.43
Estrogens									
Estrone (nmol/l)	1.82	33.9	0.96	63.5	47.2	0.18	33.3	46.6	0.47
E2 (nmol/l)	60.1	22.8	**0.009**	86.0	31.8	**0.007**	92.3	30.8	**0.003**
Non-SHBG-E2	77.4	28.9	**0.008**	109.	40.3	**0.007**	128.6	39.0	**0.001**
Insulin-like Growth Factor and its binding									
IGF-I (µg/l)	0.03	0.05	0.48	0.12	0.06	0.06	0.14	0.06	**0.02**
IGFBP-1 (µg/l)	-0.20	0.08	**0.02**	-0.39	0.12	**0.001**	-0.38		**<0.001**
IGFBP-2 (mg/l)	-9.68	4.24	**0.02**	-16.3	5.94	**0.006**	-17.2		**0.003**
IGFBP-3 (mg/l)	3.81	1.90	**0.05**	7.60	2.64	**0.004**	6.32	2.59	**0.02**
Insulin (IU/l)	-0.12	0.31	0.69	0.10	0.44	0.81	-0.13		0.76
Other									
Cortisol (nmol/l)	-0.02	0.01	**0.03**	-0.008	0.01	0.41	-0.02		**0.04**
Albumin (g/l)	-0.21	0.48	0.67	0.73	0.68	0.28	0.28	0.65	0.67

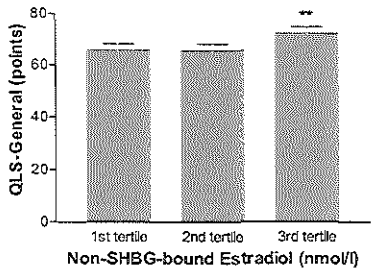
β denotes linear regression coefficient. For example: QLS-General increases 60.11 point per nmol/l Estradiol. TT=Total Testosterone, FT=Free Testosterone, SHBG = sex hormone binding globuline, Non-SHBG-T= non SHBG bound Testosterone (nmol/l), E2=Estradiol, Non-SHBG-E2 = non SHBG bound Estradiol. IGF-I = Insulin-like Growth Factor-I, IGFBP = Insulin-like Growth Factor Binding Protein

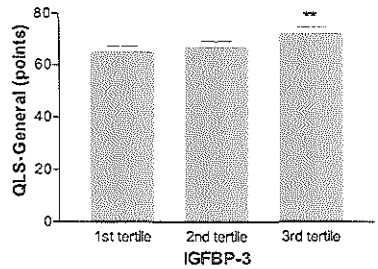
Figure 3a. Scores of the quality of life questionnaire in tertiles of serum (non-SHBG-bound) estradiol concentrations of a) the general module, b) the health module and c) the hormonal module.

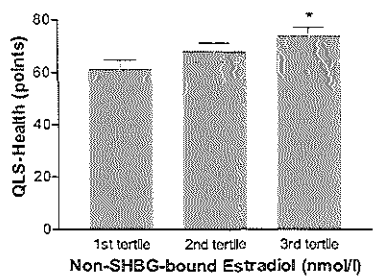
Figure 3b. Scores of the quality of life questionnaire in tertiles of serum IGFBP-3 concentrations of a) the general module, b) the health module and c) the hormonal module.

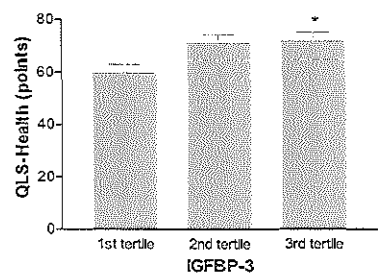
** denotes significancy of $p<0.05$ of first towards third tertile

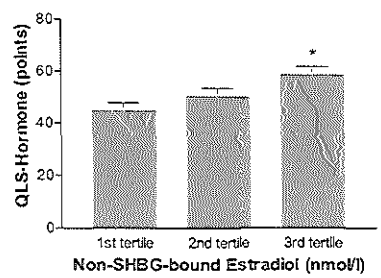
* denotes significancy of $p<0.01$ of first towards third tertile











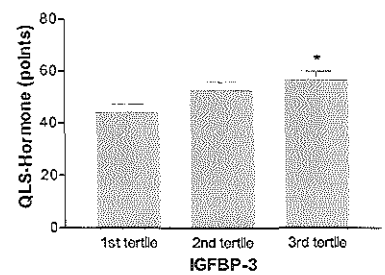
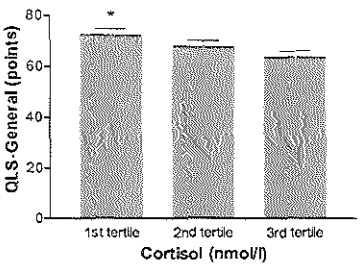


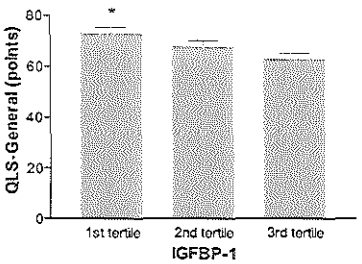
Figure 4a. Scores of the quality of life questionnaire in tertiles of serum cortisol concentrations of a) the general module, b) the health module and c) the hormonal module.

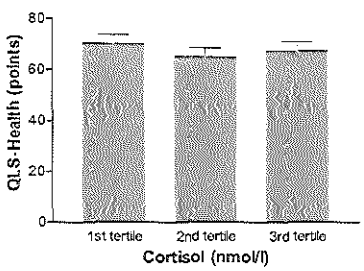
Figure 4b. Scores of the quality of life questionnaire in tertiles of serum IGFBP-1 concentrations of a) the general module, b) the health module and c) the hormonal module.

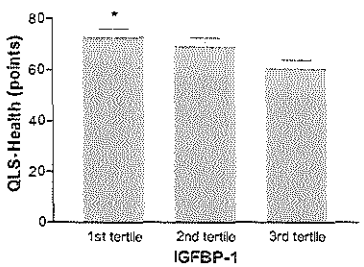
** denotes significancy of p<0.05 of first towards third tertile

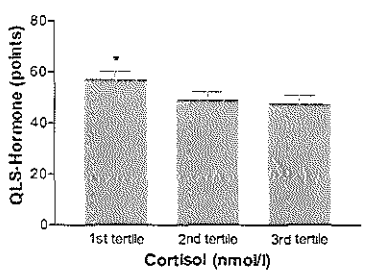
* denotes significancy of p<0.01 of first towards third tertile

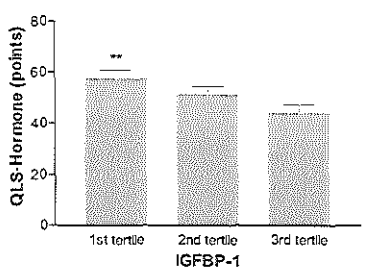












Quality of life and mortality

The health-related and hormone-related QLS modules were significant predictors of mortality (respectively OR=0.99 ± 0.003, P=0.003 and OR=0.99 ± 0.003, P=0.01). Subjects which had scores in the lowest quartile of the health-related part of the questionnaire (the least satisfied) had a significantly higher risk of mortality compared to subjects with scores in the highest quartile (respectively OR=3.81 ± 1.67 versus OR=1, P=0.002). In addition, subjects which scored in the lowest quartile of the health- as well as of the hormone-related module of the questionnaire died significantly earlier compared to subjects who scored in the highest quartile (the most satisfied) (Figure 5). QLS-General at baseline did not differentiate between deceased and survivors. Since the presence of certain diseases was related to quality of life, we adjusted for the presence of co-morbidity. Quality of life remained an independent predictor of mortality. We also adjusted for physical characteristics of aging, like muscle strength and physical performance, as well as serum hormone and albumin levels. Again, quality of life remained an independent predictor of mortality.

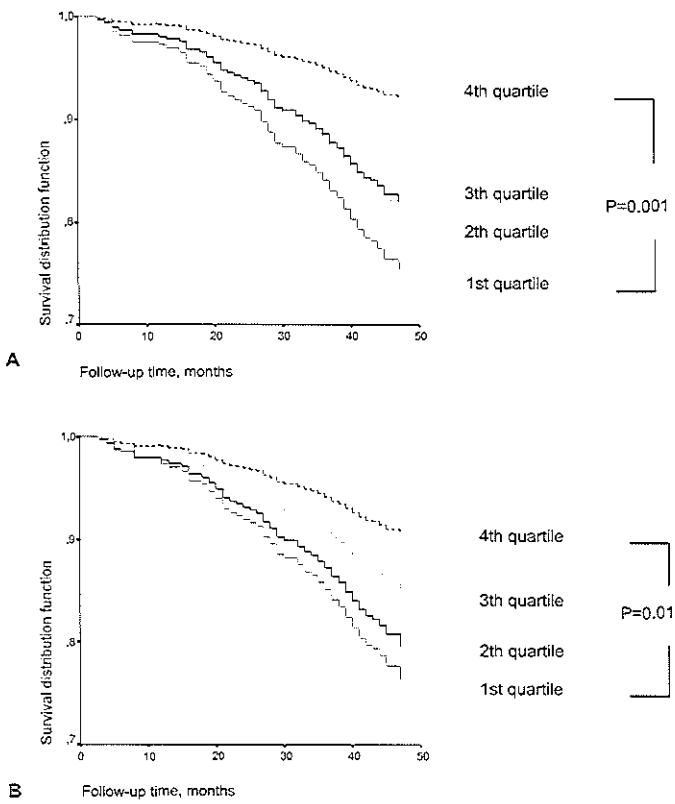


Figure 5. Cumulative survival during 48 months of quartile of the A) health module and the B) hormonal module

DISCUSSION

In a population of independently living elderly men, aged between 73 and 94 years, quality of life did not decrease with age. Several (pre)-existing diseases, however, were associated with a reduced quality of life. Maximum leg extensor strength and functional ability were strongly positively associated with the general part, the health part, as well as the hormonal part of the questionnaire on life satisfaction. Of the endocrine parameters we measured, estradiol and IGFBP-3 were strongly positively associated with general, health and hormonal related quality of life, while IGFBP-1 and cortisol were negatively related. Non-SHBG-bound testosterone was slightly, but significantly related to health-related quality of life. IGF-I was weakly positively related to the hormonal module of the quality of life questionnaire. Health- and hormonal-related quality of life are both predictors of mortality.

Psychological well-being is an important factor of successful aging (1). Quality of life (QoL) is viewed as a major outcome in its own right (25). Numerous general and illness-specific questionnaires on QoL exist (36,37). We used the questionnaire Questions on Life Satisfaction^{Modules} because it is designed to assess general, health and hormone-related QoL (24,25,38). It is a standardised, economical questionnaire in which subjective QoL is reflected by weighting the response to each item for its importance to the respondent. The questionnaire used was previously demonstrated to have high correlations with other instruments used to assess mainly psychological aspects of well-being, such as the General Well-Being Schedule-GWB (39), the Beck Depression Inventory-BDI (40) and the general symptomatic index and the scale 'Depression' of the SCL-90-R Symptom Checklist (41). In studies in healthy adult individuals correlation coefficients between the total score of health-module and these questionnaires were 0.63, -0.51, -0.31 to -0.66, respectively (25).

Summarized scores of the three modules found in our study were slightly higher than scores of male respondents aged 65 and over previously investigated by Herschbach et al. (25). Furthermore cultural differences between Germans and Dutch cannot be excluded. This difference was to be expected since this study was carried out in a selected group of independently living elderly men with relatively good physical performance, as well as cognitive function. We excluded subjects that were unable to reach the study centre independently, as well as subjects with signs of dementia. Only 30 out of the 403 men investigated had a slightly impaired score in the mini-mental state examination, between 18 and 24 points. These 30 subjects did not have different mean quality of life scores, however. The fact that we investigated a rather selected population, is also reflected in the relatively high mean score of the 'health' item in QLS-General and the 'independence of help' item in QLS-Health. In less selected populations with a broader range of functional ability, if anything, associations are likely to be stronger. The fact that quality of life did not change with age in this population, while physical well-being did decrease with age may appear as a paradox, as physical well-being and quality of life were closely related. However, in this relatively healthy group of elderly men, the oldest individuals might have a more positive outlook on life, simply due to the fact that they have became this old in a relatively healthy condition.

Various authors have associated psychological well-being with physical health, measured

by a number of organic impairments (3,42-45). Larson et al. observed that physical health seemed the most crucial factor in life satisfaction (4). Our results, with regard to physical health, are in agreement with these previously reported studies. In particular, in our study, we linked physical health, measured by a number of questions with regard to musculoskeletal or cardiorespiratory problems, to quality of life.

Few data have been reported with regard to the relations between measurements of physical functional capacity and life satisfaction in healthy elderly men. Functional independence is known as a predictive factor of life satisfaction (5). On the other hand, depressive symptoms might lead to physical disability (46,47). Not unexpectedly, scores of the modified health assessment questionnaire (MHAQ) which reports activities of daily living correlated closely with QLS. The better one can perform activities of daily living, the more satisfied one is with regard to general aspects of life. Some overlap exists between QLS-Health and the MHAQ (indeed the QLS-Health module related better with the physical characteristics than the two other QLS modules). Questions on physical performance, mobility and independence overlap with questions asked in the MHAQ. This confirms that the QLS questionnaire provides reliable information concerning these items.

Further, subjects with the best physical performance, measured objectively, have the highest life satisfaction, measured subjectively. In addition, it is interesting to note that we found a strong independent relation between muscle strength and quality of life. It might be that subjects with the highest muscle strength are better able to undertake leisure, social and physical activities and to maintain a social network. As a consequence, they may perceive a high quality of life (44). Alternatively, subjects who perceive a high quality of life, may undertake a lot, and as a consequence of this high activity level, have increased muscle strength. Importantly, the relation between muscle strength and QLS is independent of other health factors, as assessed by the medical history. Thus, physical well-being, not only measured by health factors, but also as functional ability and muscle strength, is strongly related to psychological well-being.

Hormonal activity might also be related to quality of life. In older men, the effects of androgens on mood, aspects of cognition, and sexual behaviour have not been extensively evaluated. Some studies reported improvement in the sense of well-being with testosterone supplementation (6-8). We demonstrated a weak relation between serum non-SHBG-bound testosterone levels and QLS-Health. Testosterone (mainly non-SHBG-bound) was especially related to three questions of QLS-Health (physical condition, mobility, and independence from help), suggesting that testosterone plays a role in maintaining physical function, which in turn is related to a higher life satisfaction. Controversy exists concerning the potential beneficial effects of DHEA administration on well-being in elderly men (13-15). We observed a positive relation between DHEAS and the health module of the QLS questionnaire. This relation, however, was explained through the positive relation between estradiol and QLS. To our knowledge, the strong relationship between serum estradiol levels and quality of life, has only been described in women, insofar that several studies have reported that estrogen therapy of post-menopausal women improves disease-specific aspects of quality of life, as well as more general aspects of quality of life (9-12). In our study, elderly men with the highest estradiol (E2) levels had the highest degree of life satisfaction. This relation might

be direct, through an effect of E2 on central neurotransmission in the brain (reviewed by Fink et al.(48)). The relation between E2 and life satisfaction might also be indirect by increasing physical well-being. In this study, however, the relation between E2 and QLS was independent of the physical characteristics measured.

Serum cortisol was inversely related to QLS-General and QLS-Hormone. Single measurements of total early morning cortisol levels are only of limited informative value, because cortisol is secreted in a highly episodic manner, and serum cortisol levels therefore might not represent the total 24 h production of the hormone. However, elevated serum levels of cortisol have been found to be associated with a higher degree of atherosclerosis (49,50) and increased incidence of diabetes (51,52), while they have also been associated with decreased immune function (53,54) and greater cognitive impairment (55-57). The negative influence of cortisol on the QoL might therefore represent a cumulative deleterious effect of longterm exposure of the human body to relatively high cortisol concentrations, as suggested by McEwen et al. (58).

It has been demonstrated that growth hormone (GH) treatment of adult patients with GH-deficiency increases psychological well-being (16-18). In our study serum IGF-I concentrations were found to be only weakly positively associated to the hormonal module of the quality of life questionnaire. In our study serum IGFBP-3 concentrations were strongly positively related to the general, the hormonal and especially the health related part of QLS. This relation further supports a potential role of GH on psychological well-being, since IGFBP-3 is mainly regulated by GH. Both IGFBP-1 and IGFBP-2, were significantly inversely related to mainly QLS-Health. The relation between IGFBP-2 and QLS appeared to be dependent on the physical condition and/or muscle strength, but the relation between IGFBP-1 and QLS, on the other hand, was not dependent on factors determining physical well-being. These results are in agreement with previous findings that mean IGFBP-1 levels were significantly higher in elderly subjects who reported a decreased quality of health compared to their peers (59). An explanation for this remains unknown, but was not influenced by insulin or glucose levels in our study.

The importance of defining determinants of quality of life is shown by the fact that quality of life itself turned out to be an important independent predictor of mortality. As mentioned previously, it has been reported that quality of life and depression predict physical ability in older persons. Some studies have shown that quality of life is a predictor of mortality in patients after coronary artery bypass surgery (19), with congestive heart failure (20) and with metastatic colorectal cancer (21). With regard to quality of life as predictor of mortality in relatively healthy older populations, as far as we know, only one study has been described (22). In our population of elderly men, we demonstrated that, independent of age, subjects with the lowest life satisfaction at baseline had a significant higher risk of mortality in the following four years, compared to subjects with the highest life satisfaction. In addition, we demonstrated that this significant higher risk was already present after a few months (Figure 5). Subjects with a high life satisfaction might be in good physical shape and in good health, which in turn might be the reason of an increased chance of survival. However, after adjustment for physical performance, disability and muscle strength as well as the presence of diseases in our population, life satisfaction

remained an independent predictor of mortality. Also, subjects with a higher life satisfaction might have a better social environment, which might give the support needed to maintain a good health and physical functional status. However, in our study the general part of the quality of life questionnaire, which includes questions concerning family, friends, partner and leisure time was not predictive of mortality.

In summary, in elderly men the presence of several health problems is associated with a reduced quality of life. Irrespective of the health status, physical and psychological well-being are strongly related. Muscle strength and functional ability appear to be important predictors of quality of life in independently living elderly men. In this same population, serum estradiol and IGFBP-3 levels were powerful positive predictors, while fasting early morning cortisol and IGFBP-1 levels turned out to be independent negative predictors of quality of life. Low quality of life itself is a predictor of mortality. These data, although collected in a cross-sectional study indicate that exercise in order to improve muscle strength and/or functional ability, but perhaps also hormonal intervention which aims at increasing estradiol bio-availability and/or IGFBP-3 levels might further improve quality of life in elderly independently living men. The importance of defining determinants of quality of life is shown by the fact that quality of life itself is an important independent predictor of mortality.

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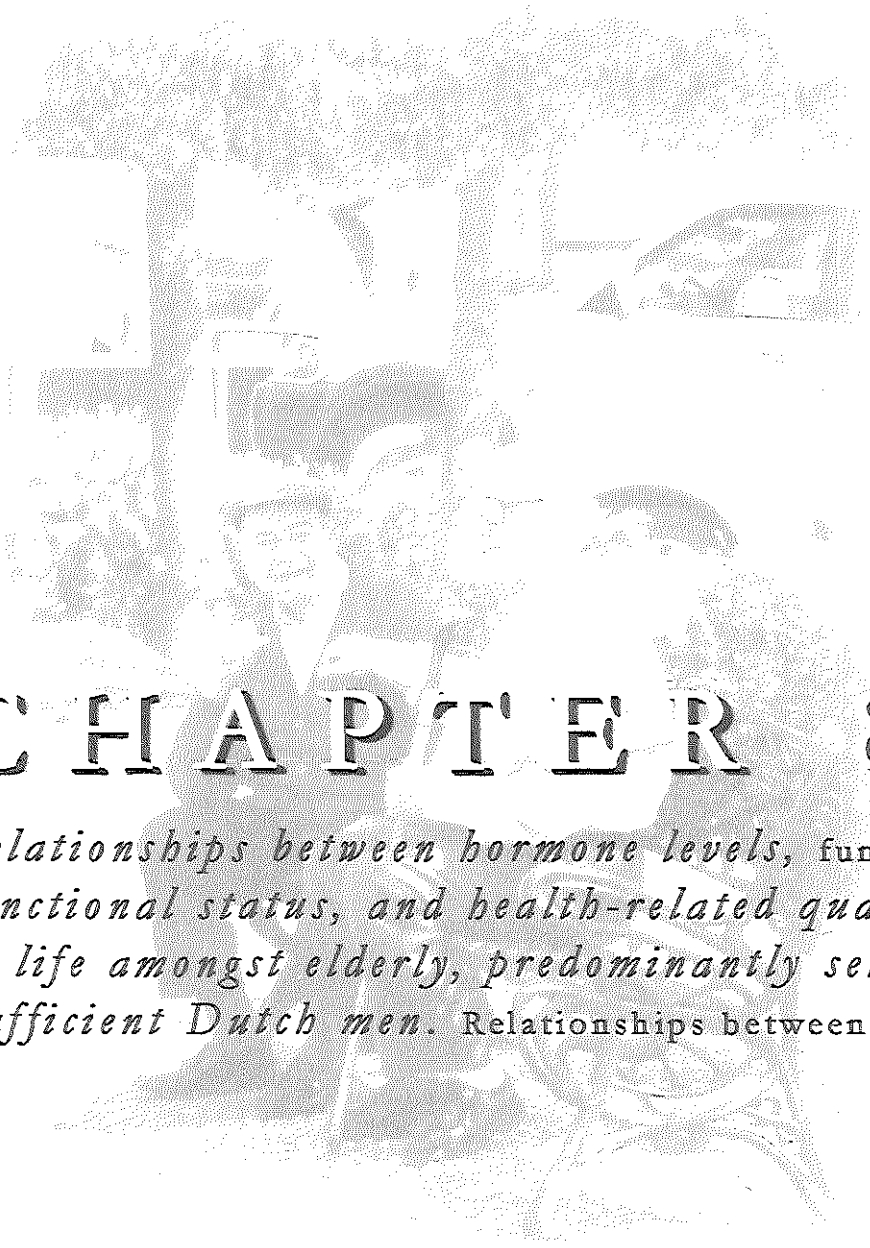

CHAPTER 8

ı men. *Relationships between hormone levels,* functional st
levels, *functional status, and health-related quality* of l
uality *of life amongst elderly, predominantly self*-sufficie
:ly self-*sufficient Dutch men.* Relationships between hormone

ABSTRACT

Background "Successful aging" has been used to differentiate between normal aging and a more desirable form of aging. One aspect of successful aging is a better health-related quality of life (HRQOL).

Objective To map the relationships between HRQOL, various physical and psychological characteristics and hormone levels.

Subjects 244 men aged 77-95 years who had been living independently in the community four years earlier.

Methods Interviews and physical examinations to collect data on hormone levels, muscle strength, physical performance, activities of daily living (ADLs), cognitive function, loneliness, and HRQOL (EQ-5D instrument). Linear regression analysis was used to examine the relationships between these parameters.

Results Despite various associations on bivariate analysis, HRQOL was associated with only ADL, loneliness and serum IGFBP-3 and testosterone levels on multiple regression. ADLs and loneliness in turn were both associated with physical performance level.

Conclusions Physical performance is associated with HRQOL, exerting its influence along both the physical and psychological pathways. The challenge remains as to whether or not physical performance can be improved well enough using exercise therapy to have a substantial impact on HRQOL, given problems with dropout and motivation. Improvements to HRQOL may be possible by hormone therapies and by social therapy to reduce loneliness.

INTRODUCTION

A differentiation has been made between normal aging and successful aging (1) and the definition of normal plays a pivotal role in this discussion. If normal is viewed as that which is *usually seen* as opposed to that which is *desirable*, it is clear that the aging process can be improved upon. For this reason, the term successful aging has been adopted. It is not surprising that there is growing interest in determining how the chance to age successfully can be increased. However the primary problem one faces initially is the definition of 'successful aging'. It is clear that the definition depends on one's perspective and value system, and in the literature, one can find various definitions for successful aging (2,3) . As a result, it is difficult to create one universally acceptable definition. We chose the model described by Rowe & Kahn (4) which contain the following components: a) a reduced chance of disease and disease-related handicap; b) maintenance of a high level of cognitive and physical function; and c) active engagement with life.

The main study objective was to examine the factors associated with successful aging amongst older, predominantly self-sufficient Dutch men. By means of different instruments and questionnaires, we operationalised selected aspects of Rowe and Kahn's model of successful aging. We focused on health-related quality of life (HRQOL), since HRQOL can be viewed as one important aspect of successful aging. In fact, since HRQOL cannot easily fit into only one of the components described by Rowe and Kahn, it can therefore be positioned above these three components. In addition, we studied the relationship between health-related quality of life and the factors of muscle weakness and physical performance, since for older-aged adults, the loss of muscle strength results in weakness, an important limiting factor for the chance to live an independent life. The association between muscle weakness and morbidity and mortality has been noted in the past (5). We were particularly interested in exploring the interrelationships between hormone levels, muscle strength, physical performance, and health-related quality of life.

MATERIALS AND METHODS

The men included in this study stemmed from a larger study population created in 1996 when all males 70 years or older living in Zoetermeer (Netherlands) were invited to participate (n=1585). In total 403 men (25.9%) were both eligible and willing to take part in the study. Since an important inclusion criterion was the ability to travel to a health research center on their own, a relatively healthy group of elderly men was included in the study (6). For the present study, all survivors from this original cohort were invited to participate. Official approval to conduct this study was received by the medical ethics committee of the Erasmus Medical Center Rotterdam.

All men were invited to visit a health research center to be interviewed and receive a physical examination. If necessary, the men were visited at their home. Various

demographic and clinical characteristics were studied for their association with health-related quality of life (Table 1).

Instruments

Health-related quality of life was measured using the EuroQol EQ-5D instrument (7), a widely used and validated generic quality of life instrument with 5 dimensions: mobility, self care, usual activities, pain/discomfort and anxiety/depression. Each dimension has three levels: no limitations, some limitations and severe limitations. All of the 243 (i.e., 5^3) possible health states have been valued by the general public in the United Kingdom using the so called York A-1 tariff, by means of a large-scale time trade-off study (8). These "valuations" of the health states represent utility values, an indication of their level of desirability or undesirability. The set of possible values has a range of -0.549 to 1, where 1 indicates perfect health, 0 indicates death and -0.549 indicates the worst possible health state. The utility values found in the UK study have since been validated for the Netherlands (9). In addition, the study participants were asked to describe their overall actual health state using the EuroQol visual analogue scale (VAS), where 0 represents the worst imaginable health state and 1 indicates perfect health.

The ability to perform activities of daily living (ADLs) and the satisfaction with this ability was measured using the 'Stanford Health Assessment Questionnaire' (MHAQ) as described by Pincus et al. (10). Scores range from 0 to 36, where a higher score indicates more problems with performing ADLs. Loneliness was measured using the Loneliness Questionnaire where possible scores range from 0 (not lonely) tot 11 (extremely lonely) (11). This questionnaire has previously been shown to be reliable and valid (12,13). The Mini-Mental State Examination (MMSE) was used to determine the level of cognitive function (14). Physical performance was assessed using the method described by Guralnik et al. (15). A physical performance score was determined based on balance, walking speed and the ability to rise from a chair (range: 0-12, where a higher score indicates better performance). Muscle strength, and specifically isometric leg extensor strength, was measured in Newton-meters as described by Hsieh en Phillips (16), using a Hoggan MicroFET hand-held dynamometer.

Hormone measurements were performed in the following manner. Blood samples were collected in the morning following an overnight fast. Serum was separated by centrifugation and stored at -40°C. Serum concentrations of total testosterone (TT, nmol/l), and sex hormone binding globulin (SHBG, nmol/l) were measured by RIA using commercial kits (Diagnostic System Laboratories, Sinsheim, Germany). The intra-assay CVs were 8.1% and 3.0% respectively. The inter-assay CVs were respectively 10.5% and 4.4%. Total IGF-I was determined by a IGFBP-blocked RIA in the presence of large excess of IGF-II (17). Intra- and interassay CVs were 1.6% and 6.4% respectively. IGFBP-1, IGFBP-2, and IGFBP-3 were measured using in-house RIAs with intra-assay CVs of 3.4%, 2.9% and 1.9% and interassay CVs of 8.1%, 10.3% and 9.2% respectively (17).

Statistical analyses

All characteristics of the men were studied for their association with health-related quality of life (see Table 1). Bivariate and multivariate analyses were performed using linear regression analysis where HRQOL (i.e., EQ-5D utility score) was the dependent variable. Multivariate analysis was used to establish which patient characteristics were statistically significantly associated with HRQOL after adjustment for other characteristics. Both stepwise and best subsets analysis were used to create the final statistical models and a p-value of 0.05 was applied as a guideline for inclusion into the model. Associations between patient characteristics and each of the five dimensions of the EQ5D scale were analyzed by means of bivariate analysis (Mann-Whitney U test, Kruskal-Wallis test, Kendall's tau where appropriate) and stepwise multivariate logistic regression analysis (binary and ordinal logistic regression). Correlation between the EQ5D utility scores and the EuroQol VAS scores was examined using both the Pearson and the Spearman correlation methods. In subsequent analyses we examined the factors associated with the outcomes of loneliness and ADLs in a manner similar to that described above for the HRQOL. Data were analyzed using SPSS version 8.0.

RESULTS

Of the 403 men from the study in 1996, 244 men (60%) participated in the survey. The remaining 159 men did not participate for the following reasons: 75 (19%) were deceased; 21 (5%) had moved, and 63 (16%) were not willing to participate, their reasons including "too sick" (n=24), "no reason" (n=22), interview too taxing (n=5), demented (n=4), wife too sick (n=4), and other reasons (n=4). Most participants were seen at the research center (215, 88%), the others interviewed at home. Characteristics of the study population are found in Table 1. The average age was 81 years (range: 77-95 years). Most men were married and still living independently at home without assistance. The average score for loneliness was 2.5, and approximately 40% of the men were found to be at least moderately lonely (i.e., score ≥ 3).

Quality of life based on EQ5D instrument

The average EQ-5D utility score was 0.80 and the average EuroQol VAS was 0.76. In a general population of similarly aged men, Kind et al reported an average EQ-5D utility score of 0.75 and an average EuroQol VAS score of 0.73 (18). The HRQOL seen in this population was therefore higher than that seen in the general population. The correlation between the EQ-5D utility score and the EuroQol VAS score was 0.394 ($p < 0.0001$).

Most men reported having little or no problems with each of the five EQ-5D dimensions (Table 2). In fact, 30% of the men scored the highest EuroQol utility score of 1, indicating no problems whatsoever with any of the dimensions. Problems with mobility were most frequent in this study group and increased with age (Table 2). Interestingly, most men reported no problems with self-care and daily activities. The

only statistically significant association between age and limitations was seen with mobility (Kendall tau = 0.130, $p<0.05$).

Table 1. Description of the study population (n=244)

	Mean (SD),	Range
Age in years	81.2 (3.3)	77-95
Marital status (n, %)		
Married	182 (75%)	
Single, never married	7 (3%)	
Divorced	7 (3%)	
Widowed	48 (20%)	
Living situation (n, %)		
Living independently, without assistance	101 (41%)	
Living independently, with assistance	84 (34%)	
Service center	41 (17%)	
Seniors flat	8 (3%)	
Home for seniors	10 (4%)	
Muscle strength (Newton-meters)*	106.04 (23.05)	
Physical performance score (points)	8.28 (3.16)	
MMSE score (cognitive function)*	26.82 (2.74)	
Activities of Daily Living score (points)	13.00 (6.44)	0-36
Loneliness score	2.49 (2.43)	0-10
Not lonely (score of 0-2) (n, %)	142 (57%)	
Moderately lonely (score of 3-8) (n, %)	96 (39%)	
Extremely lonely (score of 9-11) (n, %)	6 (3%)	
EQ-5D utility score	0.80 (0.21)	
EuroQol VAS score	0.76 (0.14)	

NB: Means with standard deviations in brackets are shown unless otherwise indicated. * - One observation missing.

Table 2. Frequency of problems with the five EQ-5D dimensions per age group * - $p < 0.05$

EQ-5D dimension	75-79 yrs (n = 96)	80-84 yrs (n = 106)	85-89 yrs (n = 36)	90-95 yrs (n = 6)	Total (n = 244)	Kendall tau correlation coefficient
Mobility	43%	54%	61%	67%	50%	0.130*
Self-care	6%	10%	14%	17%	9%	0.050
Daily activities	11%	14%	17%	17%	13%	0.047
Pain/discomfort	38%	41%	34%	50%	36%	-0.016
Anxiety/depression	22%	17%	14%	17%	18%	-0.055
Mean EQ-5D utility score (SD)	0.82 (0.20)	0.80 (0.23)	0.80 (0.16)	0.78 (0.05)	0.81 (0.21)	
Mean EuroQol VAS (SD)	0.77 (0.14)	0.75 (0.15)	0.76 (0.14)	0.63 (0.12)	0.76 (0.14)	

Association between HRQOL and personal characteristics

Table 3 shows the results of linear regression analyses involving the EQ-5D utility score and the EuroQol VAS score. Age was not statistically significant associated with the EQ-5D utility score but was associated with the EuroQol VAS score. Various factors were associated with both EQ-5D utility score and EuroQol VAS score (Table 3). Following multivariate analysis, only the ADL score (beta=-0.019, 95%CI: -0.022, -0.015; p<0.001) and loneliness (beta=-0.016, 95%CI: -0.024, -0.017; p<0.001) remained in the model using the EQ-5D utility score as the dependent variable (R-squared, 40%).The majority of the predictive ability in this model was derived from the ADL score (i.e., 37% of the R-square of 40%).

To explore the mechanisms underlying the associations between HRQOL, ADLs and loneliness, we examined their correlations with the five EQ-5D dimensions. The ADL score was statistically significantly associated with all five dimensions (range of Kendall tau correlation coefficients: 0.246-0.639). In contrast, loneliness was statistically significantly associated with mobility (Kendall tau=0.174, p<0.01), self-care (Kendall tau=0.206, p<0.01) and anxiety/depression (Kendall tau=0.267, p<0.01).

Table 4 shows the correlations between the EQ-5D results and the levels of the hormones that were measured. Insulin-like growth factor (IGF) and IGF binding protein 3 were the only hormones associated with both the EQ-5D utility score and VAS scores. Total testosterone was associated with only one of the two EQ-5D scores. When the individual EQ-5D dimensions were examined, various associations were statistically significant (see Table 4).

Table 3. Relationships between health-related quality of life (EQ-5D) and personal characteristics (univariable linear regression analyses)

	EQ-5D utility		EuroQol VAS	
	Beta coefficient	S.E.	Beta coefficient	S.E.
Age (in years)	-0.003	0.004	-0.007*	0.003
Marital status (relative to married men)				
Unmarried	0.019	0.080	-0.022	0.055
Divorced	-0.023	0.080	-0.045	0.055
Widowed	-0.036	0.034	-0.012	0.023
Living situation (relative to men living independently without assistance)				
Living independently with assistance	-0.067*	0.029	-0.056**	0.020
Service center	-0.011	0.370	-0.051	0.025
Seniors flat	-0.146*	0.073	-0.116*	0.050
Home for the elderly	-0.276***	0.066	-0.196***	0.045
ADL score (points)	-0.020***	0.002	-0.010***	0.001
Loneliness score	-3.300***	0.722	-0.011**	0.004
Physical performance (points)	0.030***	0.004	0.019***	0.003
Muscle strength (in Newton-meters)	0.003***	0.001	0.002***	0.0004
Cognitive function (using MMSE)	0.013**	0.015	0.007*	0.003

NOTE: A positive coefficient indicates a better quality of life. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Problems with activities of daily living

Subsequently, we studied the characteristics associated with ADL. Excluding marital status, all characteristics were associated with ADL problems (Table 4). The final model consisted of physical performance (beta=-1.05, 95%CI: -1.27,-0.83; p<0.001), muscle strength (beta=-0.056, 95%CI: -0.086, -0.026; p<0.001), living independently with assistance (beta=1.509, 95%CI: 1.303, 2.715, p<0.05) and living in a home for seniors (beta=5.805, 95%CI: 2.945, 7.945; p<0.001) This model explained 54% of the variation in ADL scores (R-square=54%), owing particularly to the presence of physical performance in the model (R-square=47%).

Table 4. Correlations between hormone levels and EQ-5D health-related quality of life scores and dimensions (Values in bold are Pearson correlation coefficients for utility and VAS scores, and Kendall tau correlation coefficients for the EQ-5D dimensions)(p-values shown in italics)

	IGF-1 (ng/l)	IGFBP-1 (ng/l)	IGFBP-2 (µg/l)	IGFBP-3 (µg/l)	Total Testosterone (nmol/l)
EQ-5D utility score	**0.144**	**-0.059**	**-0.077**	**0.194**	**0.091**
	0.024	*0.358*	*0.233*	*0.002*	*0.158*
EQ-5D VAS score	**0.129**	**-0.105**	**-0.085**	**0.223**	**0.286**
	0.044	*0.101*	*0.187*	*0.001*	*<.0001*
EQ-5D dimensions					
1. Mobility	**-0.029**	**0.073**	**0.124**	**-0.074**	**-0.058**
	0.581	*0.167*	*0.018*	*0.158*	*0.269*
2. Self-care	**-0.104**	**0.117**	**0.159**	**-0.103**	**-0.096**
	0.047	*0.025*	*0.002*	*0.050*	*0.068*
3. Usual activities	**-0.149**	**0.042**	**0.034**	**-0.178**	**-0.018**
	0.005	*0.424*	*0.517*	*0.001*	*0.726*
4. Pain and discomfort	**-0.084**	**0.002**	**-0.020**	**-0.111**	**-0.150**
	0.107	*0.976*	*0.706*	*0.033*	*0.004*
5. Anxiety and depression	**-0.047**	**0.091**	**0.091**	**-0.116**	**-0.090**
	0.370	*0.080*	*0.081*	*0.027*	*0.086*

Insulin-like Growth Factor-1 (IGF-I), Insulin-like Growth Factor Binding protein (IGFBP)-1,-2,-3

Loneliness

We also examined the factors associated with loneliness (Table 5). Widowers, men showing poor physical performance, and men with less muscle strength were on average lonelier than other men. The final model consisted of widower (beta=1.637, 95% CI: 0.752, 2.389, p<0.001), physical performance score (beta=-0.220, 95% CI: -0.322,-0.118, p<0.001), and age (beta=-0.113, 95% CI: -0.209,-0.017, p<0.05). This model explained 13.4% of the variation in loneliness scores.

Table 5. Relationships between problems with activities of daily living and patient characteristics (bivariate analyses using linear regression analysis)

	Problems with activities of daily living		Loneliness	
	Beta coefficient	Standard error	Beta coefficient	Standard error
Age in years	0.41***	0.12	0.013	0.47
Marital status (relative to married)				
Single	-2.54	2.49	1.055	0.901
Divorced	0.75	2.49	1.484	0.901
Widowed	0.45	1.05	1.662***	0.380
Living situation (relative to men living independently without assistance)				
Living independently with assistance	3.94***	0.86	-0.031	0.359
Service center	1.93	1.08	0.282	0.450
Seniors flat	7.67***	2.15	0.999	0.893
Home for the elderly	11.92***	1.94	1.024	0.806
Loneliness score	0.45**	0.17	n/a	n/a
Activities of daily living	n/a	n/a	0.063**	0.024
Physical performance score	-1.42***	0.09	-0.172***	0.048
Muscle strength (Newton-meters)	-0.15***	0.02	-0.020**	0.007
MMSE score (cognitive function)	-0.37*	0.15	-0.070	0.057

NOTE: A positive coefficient indicates increased problems with performing activities of daily living. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Physical performance

Lastly, we analyzed the physical performance score as a dependent variable. The final model consisted of age (beta= -0.355, 95%CI: -0.480, -0.229; p=<.0001), IGF BP-3 (beta=0.035, 95%CI: 0.012, 0.058; p=0.003), and total testosterone (beta=0.127; 95%CI: 0.003, 0.251; p= 0.04).

DISCUSSION

The definition for successful aging is subjective, influenced by cultural and individual values. In our study we avoided the issue of defining successful aging by focusing on aspects which cannot be seen as arguable. All would agree that a better HRQOL, reduced loneliness, and reduced problems with performing ADLs is desirable, regardless of one's preferred definition of successful aging. We studied the factors associated with HRQOL and found that problems with ADLs and loneliness were associated with a lower HRQOL.

We found that most men in our study lived independently, with or without assistance (76%). Interestingly, the Longitudinal Aging Study Amsterdam (1998) involving older people living in the Netherlands reported that 93% of men aged 77-92 years lived independently, a frequency much higher than what we found. This study also reported that 28% of the men living independently received no domestic help and 87% received no assistance regarding self-care, in contrast to the 83% and 70% we found in our study (19).

In this study, we found that 42% of the men were at least moderately lonely, in concordance with previous studies involving Dutch older people where frequencies ranging from 32% to 63% and 71% have been reported (20).

HRQOL

The positive association between problems with performing ADLs and lower HRQOL has been noted before (21). Regarding the positive association between loneliness and reduced HRQOL, Mullins et al. found that a poor self-reported health status was associated with greater level of loneliness (22).

ADL

Physical performance, muscle strength and living situation were associated with ADL. Guralnik et al found that persons with higher physical performance scores had fewer problems with mobility and with ADLs (15).

Loneliness

We found that being a widower, increased problems performing ADLs, reduced physical performance, and reduced muscle strength were associated with increased loneliness. In a previous study, men without a partner showed increased levels of loneliness (20). The explanation given for this finding involved the quantity and quality of social relations. Older people without a partner do not only lack another person who can represent an important source of love and support, but they lead a more isolated life as a result, participate less often in social activities, feel left out more often and can rely on fewer people in times of need.

The association between physical performance and loneliness has been noted before (20) where a negative association between functional status and loneliness was reported. One solely psychological explanation is that poor health leads to a general feeling of dissatisfaction which in turn manifests itself in loneliness. A different explanation is that the restrictions resulting from poorer physical performance mean that older people with functional problems are less able to improve and maintain their social network in order to meet their needs and desires. It has been suggested that the limited possibility to perform practical tasks independently and the desire not to ask others continually for help exacerbates the feelings of loneliness (20). It has been reported that persons with more social ties show fewer functional problems and this association appears stronger in men than in women (23). When men receive more emotional support, less loneliness is

experienced, more practical support is received, and a reduced risk of mortality is observed (24).

Physical performance

Impairments at an older age are not irreversible aspects of the aging and disease, and instead they can be reduced and maybe even prevented (25). Moreover amongst those seniors without handicaps, objective measures of 'physical performance' are very predictive of later handicaps. Various studies have shown that physical performance can be improved (15). Moderate levels of exercise such as walking at a normal pace appear to result in similar effects regarding physical performance to those seen following more energetic exercise forms such as walking fast (4). However, while reduction of impairment can be achieved by exercise, the activity of exercise is difficult to integrate into the lives of seniors and the dropout rate of exercise programs is very high (25). An alternative is the use of medicines to improve physical performance (25). We found that a number of different types of hormones were associated with physical performance. However, one might question the real significance of these associations. That is, does the presence of such associations indicate that hormone therapy would improve physical performance? Such an intervention needs to be evaluated using a randomized controlled study design.

Improvements in physical performance can act to reduce problems with ADL as well as reduce loneliness. However, the association between physical performance and loneliness was relatively weak though statistically significant, meaning that loneliness can only somewhat be ameliorated by improving physical performance. Since this is the case, it is useful to consider other means of reducing loneliness. Linnemann & Leene have stated that seniors mainly seek support in their existing primary network when feelings of loneliness arise. Many people become confronted with a steadily shrinking network of contacts. Moreover, it is not possible to replace old contacts with new ones. An important task is to ensure that physical limitations do not present an obstacle against participation in an active social life since the removal of difficulties faced in taking part in social activities is an important success factor (26). How can this problem with loneliness be helped? One possibility would be to arrange for more home visits, for example by health care workers, in a sort of preventive care effort. Interest from a health care perspective would lie in the rationale that reduced loneliness will eventually lead to reduced need for health care. A longitudinal study is necessary to examine the effectiveness of such an intervention (26). Another possibility would be the creation of opportunities where seniors could meet other seniors, for example the organization of excursions such as sightseeing trips, sporting events, and theatre performances. One variant would be a sort of matchmaking initiative, where seniors first would be asked to identify their interests and then subsequently would be "matched" with other seniors reporting the same interests. A more medical approach would involve the use of medication for depression, which may lead to reduced loneliness (27).

Hormones

It is well-known that growth hormone deficient as well as hypogonadal adults have an impaired quality of life compared to controls (28). In the elderly, the association between a decrease in serum hormone levels and quality of life is much less clear (29). Only few studies have investigated the effect of hormone replacement on quality of life in the elderly (30). In this study serum IGFBP-3 levels, which are a representative of 24-hour growth hormone secretion, as well as IGF-I were strongly positively related with health-related quality of life. How will growth hormone exert its potential effect on quality of life? Although IGFBP-3 levels were also positively related to physical performance, the relation between IGFBP-3 and HRQOL seemed not to be determined by physical performance. The significant relations between IGFBP-3 and the dimensions of the EQ-5D utility may give some other indications. In addition, a strong positive association was found between serum testosterone levels and the EuroQol visual analogue scale, which is a measurement of a more subjective and personal quality of life experience. It could be hypothesized that higher testosterone levels are related to a better functional ability, and therefore are related to a better health-related quality of life. However, the relation between testosterone and HRQOL was independent of physical performance and muscle strength. The presence of disease itself may also be associated with decreased testosterone levels.

Study limitations

As noted earlier, all men included in this study originally had to fulfill a number of criteria to be included in this study, such as living independently in the community and the absence of dementia. As a result, some of our findings are not generalisable to all type of older people (e.g., average physical performance). Nevertheless, some results are still relevant. The associations we found between ADL, loneliness and HRQOL corresponded with what one could have expected beforehand. Had a random sample been examined, it is quite likely that these associations would have been even stronger (10,22).

Some of the men invited to participate in the study were not able to participate, for example because they were too sick. Undoubtedly the ADL scores and HRQOL scores would have been poorer had these men participated in the study. However, their presence in the study would likely have made the associations we found even stronger.

Given the cross-sectional nature of this study, any conclusions regarding causal relationships therefore depend on plausibility and assumptions.

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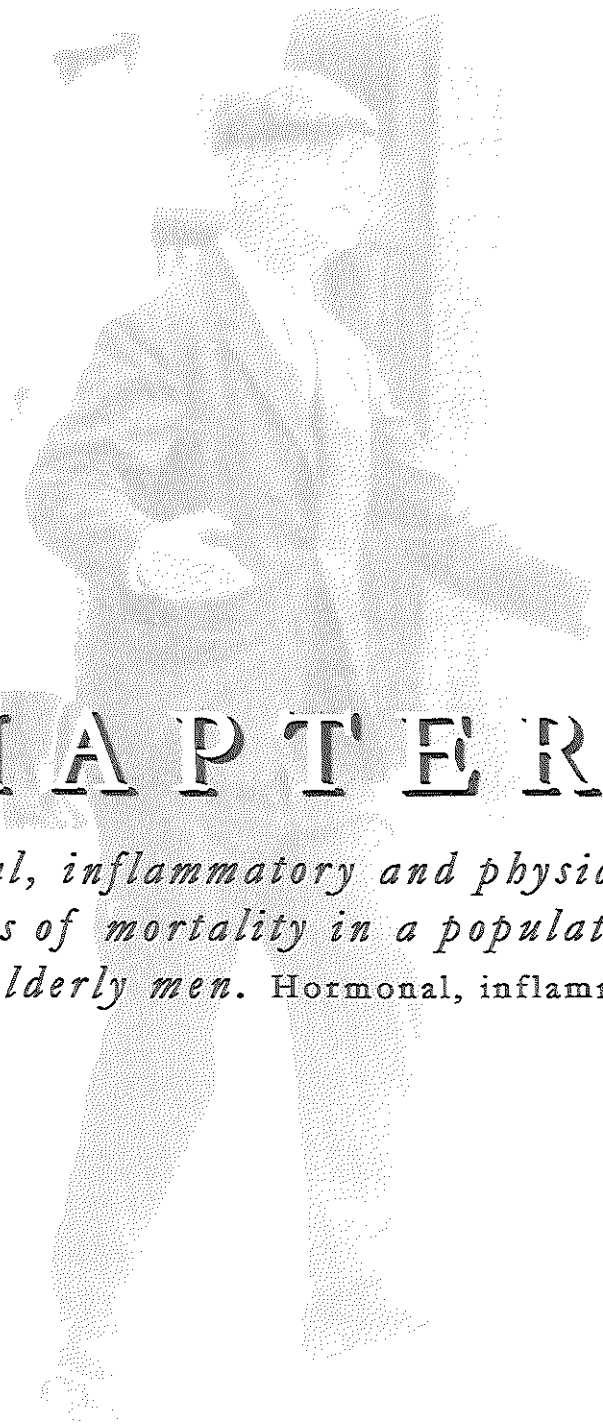

CHAPTER 9

y men. *Hormonal, inflammatory and physical* predictors c
hysical *predictors of mortality in a population of* healthy
:ion of *healthy elderly men.* Hormonal, inflammatory and phys

ABSTRACT

Background We investigated the relative importance of several physical, endocrine and inflammatory parameters as potential predictors of mortality in a group of 403 relatively active, non-disabled independently living elderly men of around 80 years of age.

Methods The physical characteristics measured included a measure of physical performance, muscle strength, bone mineral density of total body and proximal femur and body composition. Endocrine parameters included serum insulin-like growth factor (IGF)-I and its binding proteins (IGFBP) -1, -2 and -3 concentrations, testosterone, cortisol, DHEA(S), free T4, (reverse) T3 and albumin concentrations. Inflammatory parameters C-Reactive Protein (via a high-sensitive assay) and serum interleukin-6 were measured. Intima-media thickness (IMT) of the carotid artery was also measured. Mortality was registered in the subsequent four years.

Results Four years after the initial investigation, 75 subjects had died (19%). Age, smoking, co-morbidity and IMT of the carotid artery were powerful predictors of death. However, in addition and independent of them a number of other factors and parameters emerged to be of importance in the prediction of death. High physical performance, muscle strength and bone mineral density were associated with a lower risk of mortality (RR=0.71 (0.57 – 0.89), RR=0.70 (0.55 – 0.90) and RR=0.74 (0.57 – 0.95) respectively). Of the hormones, high serum IGFBP-1, free T4 and T3 concentrations all predicted mortality (RR=1.29 (1.06 – 1.58), RR=1.27 (1.01 – 1.60) and RR=1.30 (1.02 – 1.60) respectively). Finally, low serum albumin and high C-reactive protein and interleukin-6 levels were associated with a higher mortality rate (RR=0.68 (0.53 – 0.86), RR=1.33 (1.07 – 1.67) and RR=1.74 (1.35 – 2.24) respectively). With the seven most significant parameters 78% of subjects could be correctly identified with respect to alive or death after four years (area under the ROC curve of 0.78).

Conclusions To the best of our knowledge, this is the first study in which apart from the well-known conditions like smoking, co-morbidity and atherosclerosis, the relative importance of a range of potential physical, endocrine and inflammatory predictors of mortality were addressed in one single study. It appeared that several endocrine and inflammatory factors are equally important as indicators of physical functional ability, like bone mineral density and muscle strength, in predicting four year mortality in the elderly, independent of co-morbidity. Importantly, the concentrations of these factors were within the normal range.

INTRODUCTION

There is considerable variation in biologic aging in healthy individuals, with some persons exhibiting extensive decline in physiological functions with age and others little or none. This suggests that there are more and less "successful" patterns of aging (1). Genetic factors, lifestyle, and societal investments in a safe and healthy environment are important determinants of successful aging (1). Traditionally, aging has been considered physiological and unavoidable. In recent years it has become evident that it may not be necessary to accept the grim stereotype of aging as an inevitable process of decline and loss (2,3). Prevention of loss of physical functions can be achieved by exercise (3), while hormone replacement with growth hormone (4-6) and testosterone (7) have been demonstrated to increase muscle mass, and in some instances increase muscle strength in elderly individuals. To be informed about one's individual condition and to select those who may benefit from intervention, the use and knowledge of modifiable parameters that predict survival or mortality may be useful. It is well-known that co-morbidity and advanced atherosclerosis are indicators of mortality (8). Although several studies indicate the predictive value for mortality of e.g. physical and inflammatory parameters, to date no study has been performed in which a broad spectrum of potential parameters was studied in a single elderly population.

In the present study we investigated the relative importance of a range of possible predictors of mortality in a group of 403 non-disabled independently living elderly men. Functional ability, hormone concentrations of the gonadal, adrenal, somatotropic and thyroidal axis as well as inflammatory parameters were studied and related to mortality in the subsequent four years.

METHODS

Subjects

A single-center cohort study was conducted in 403 independently living men, aged 70 years and higher. At baseline, 1567 men 70 years and older were invited of which the addresses were drawn from the municipal register of a medium sized town in the Netherlands. A total of 886 men did not respond to the mailed invitation in which it was mentioned that only subjects who lived independently and had no severe mobility problems could participate. Eventually 403 men participated (25.7%). The main reason not to participate among the respondents was because they were currently under the care of a medical specialist or general practitioner (28%), while 16% was excluded on the basis of physical (10%) or cognitive (6%) problems. Participants signed an informed consent. The study was approved by the Medical Ethics Committee of the Erasmus University Hospital Rotterdam.

At baseline a 21 item medical history was obtained by a structured questionnaire, according to the following groups: musculoskeletal impairments (including arthrosis and fractures); cardiovascular impairments (including symptoms or treatment of angina

pectoris, congestive heart failure, hypertension, arythmias, myocardial infarction, cerebrovascular accidents and shortness of breath); prostate problems (hyperplasia and cancer); other malignancies; endocrine disorders (diabetes mellitus and thyroid disease); and other conditions (dizziness and disturbed vision which impairs mobility). We divided the cohort in five groups according to the number of complaints or diseases present as indicated by the 21 item medical history (from 0 till ≥ 5). None of the subjects was treated for systemic infectious, inflammatory or malignant disorders at the time of the investigation.

A number of participants were taking medications for hypertension (n=96), angina pectoris or a myocardial infarction more than six months ago (n=85), mild congestive heart failure (n=28), chronic obstructive pulmonary disease (n=40), diabetes (n=28), and hypercholesterolemia (n=7).

A complete physical examination was performed.

Four years after the initial investigation 75 men (19%) had died (8 in the first year, 16 in the second year, 21 in the third year and 30 men in the fourth year).

Physical characteristics

Physical Performance

Lower extremity function, or physical performance, was assessed as described by Guralnik et al.(9), including measurements of standing balance, walking speed and ability to rise from a chair. A summary performance scale was created by summing the category scores for the walking, chair stand, and balance test, which ranged from 0 (worst performance) to 12 (best performance). Mean scores of the three tests as well as of the summary performance scale measured in this study were similar to those reported in subjects of the same age group investigated by Guralnik (9).

Activities of daily living

Self-reported disability or satisfaction in performing activities of daily living was assessed by a self-administered questionnaire modified from the Stanford Health Assessment Questionnaire as described by Pincus et al (10). A score of 8 points was obtained if the participants reported no problems in activities of daily living, needed no help and when there was no difference compared with the situation 6 months ago. A minimum score of 0 was given if participants improved compared to 6 months previously, while a maximum score of 36 points was assigned to participants who reported severe problems in activities of daily living, needed help and had more problems in activities of daily living compared to 6 months ago. The lower the points measured in this ADL score, the lower the degree of disability. The Mini-Mental State Examination was used to obtain and estimate cognitive function (11).

Muscle Strength

Isometric leg extensor strength was measured as described by Hsieh and Philips using the Hoggan MicroFET hand held dynamometer (12,13). The measurement requires that the participant, in a seated position, pushes with maximal strength to the dynamometer, which is held at the tibia. The investigator holds the dynamometer in the hand with an extended arm and pushes back until the breaking point is reached. To

obtain one measure of leg muscle strength, "maximum leg extensor strength" was defined as the maximum strength for the right or left leg in a position of 120°. Statistical analyses were based on the physical unit measurement, moments (N*m), obtained by multiplying the maximum strength (in Newton) and the distance of the dynamometer to the knee joint (in meters).

Isometric grip strength (IGS) was tested using an adjustable hand-held dynamometer (JAMAR dynamometer) in the non-dominant hand (14). Each test was repeated three times and the average, expressed in kilo ponds (kp), was used in the analysis.

Bone mineral density and Body Composition

Total body bone mineral density was measured using dual energy x-ray absorptiometry (DXA, Lunar, Madison, WI), as were hip bone mineral densities at the femoral neck, trochanter and Ward's triangle. In addition total lean body mass and fat mass were measured (15,16). Quality control including calibration was performed routinely every morning for DXA, using the standard provided by the manufacturer.

Measures of carotid atherosclerosis

To determine carotid artery intima-media thickness as a quantitative measure of generalized atherosclerosis (17), ultrasonography of the left and right common carotid artery and the bifurcation was performed with a 7.5-MHz linear array transducer (ATL Ultramark IV, Advanced Technology Laboratories). A careful search was performed for all interfaces of the near and far wall of the distal common carotid artery and the far wall of the carotid bifurcation (18). The actual measurements of the intima-media thickness were performed off line as described previously (19).

The common carotid artery, carotid bifurcation and internal carotid artery were also evaluated for the presence (yes / no) of atherosclerotic lesions on both the near and far wall of the carotid arteries. Plaques were defined as a focal widening relative to adjacent segments, with protrusions into the lumen composed of only calcified deposits or a combination of calcification and non-calcified material (20). Also the total number of plaques was calculated.

Hormone measurements

Blood samples were collected in the morning after an overnight fast. Serum was separated by centrifugation and stored at -40°C. The period of storage at -40□C varied from 0 till 5 months. Total Insulin-like Growth Factor (IGF) -I was determined by a IGF-Binding Protein-blocked RIA in the presence of large excess of IGF-II as described. (21) Intra- and interassay CVs were 1.6% and 6.4% respectively. IGFBP-1, IGFBP-2, and IGFBP-3 were all measured with in-house radioimmunoassays (RIA) as described previously with intra-assay coefficients of variation (CV) of 3.4%, 2.9% and 1.9% and inter-assay CV of 8.1%, 10.3% and 9.2%, respectively (21). Insulin was measured by a commercially available RIA (Medgenix Diagnostics, intra-assay and inter-assay CV: 8.0% and 13.7%).

Albumin (g/l) was measured by photometry using a commercial kit (ALB, Boehringer, Mannheim, Germany). Serum concentrations of total testosterone (TT, nmol/l), and sex

hormone binding globulin (SHBG, nmol/l) were measured by RIA using commercial kits (Diagnostic System Laboratories, Sinsheim, Germany). The intra-assay CV were 8.1% and 3.0% respectively. The inter-assay CV were respectively 10.5% and 4.4%. In addition, serum concentrations of estrone (E1, nmol/l), estradiol (E2, nmol/l), DHEA (nmol/l), and DHEAS (μmol/l) were also measured by RIA using commercial kits (Diagnostic System Laboratories). The intra-assay CV were 5.6%, 5.3%, 3.8%, and 2.1% respectively. The inter-assay CV were 10.2%, 8.1%, 8.6%, and 5.1% respectively. Non SHBG bound testosterone (nmol/l) was calculated according to a method described by Södergård et al.(22). Cortisol (nmol/l) and insulin (mIU/l) were measured by RIA (Diagnostic System Laboratories).

Thyroid-stimulating hormone (TSH) was measured using an immunometric technique (Amerlite TSH-30, Ortho-Clinical Diagnostics, Amersham, UK). Free thyroxine (FT4), thyroxine (T4), triiodothyronine (T3), and reverse T3 were all measured by RIA (Amerlite MAB FT4 assay, Ortho-Clinical Diagnostics, Amersham, UK; (23)). Thyroid binding globulin was measured by a chemoluminiscence method. Intra- and intervariability coefficients of all the assays were below 11%.

C-Reactive Protein (CRP) concentrations were determined by a high sensitive method using a latex-enhanced immunoephelometric assay on a BN II analyser (Dade Behring, Liederbach, Germany). Interleukin-6 was measured using a commercially available immulite assay (Diagnostic Products Corporation).

Data analyses

Results are expressed, unless otherwise indicated, as mean and standard deviation with the interquartile (IQ) range. Variables which were not normally distributed were logarithmically transformed. Cox regression was used to analyse the associations under study with mortality as outcome. The relative risks and its 95% Confidence Interval (CI) are based on the hazard ratios per standard deviation increase using a Cox proportional hazards model. Multivariate analysis was used to adjust for age, as well as to assess the contribution of different independent variables to the dependent variable. Analyses were performed using Stata statistical package (StataCorp. 1997. Stata Statistical Software: Release 5.0).

RESULTS

Mean age of the study population was 77.8 yr (Standard Deviation 3.6). Baseline study characteristics are given in Table 1. 70 Subjects were current smokers, while 281 subjects were former smokers. 51 Subjects had never smoked.

General predictors of mortality

Higher age was associated with an increased risk of mortality. Subjects who had never smoked had a significantly lower risk of mortality compared to subjects who ever smoked or smoked at the moment (Table 2). A higher number of complaints or diseases was significantly related to a higher risk of mortality (Table 2). In addition, indicators of

(sub)clinical atherosclerosis such as a thicker intima-media of the carotid bifurcation as well as a higher number of plaques in the carotid artery were associated with a higher relative risk of mortality (Table 2).

Predictors of mortality; physical characteristics

Independent of age, the risk of death decreased significantly with increasing scores on the physical performance test (Table 2). More problems in activities of daily living were related to a higher risk of mortality of borderline significance (Table 2). Increasing muscle strength and bone mineral density values of the total body and the femur were, independent of age, significantly related to a lower risk of mortality (Table 2). After adjustment for age, subjects with physical performance scores (A) in the lowest quartile, as well as subjects with the lowest muscle strength (B) and the lowest bone mineral density (C), and subjects with the highest number of problems in activities of daily living died significantly earlier after the investigation compared to subjects in the highest quartiles (Figure 1 A-C).

Predictors of mortality; somatotropic hormones

High IGFBP-1 and IGFBP-2 concentrations, independent of age and insulin concentrations, predicted mortality (Table 2). After adjustment for the presence of plaques and the distensibility of the carotid artery, IGFBP-1 remained significantly related to mortality. However, after adjustment for intima-media thickness of the carotid bifurcation, IGFBP-1 no longer predicted mortality (RR=1.29 (0.94 – 1.78). Serum IGF-I, IGFBP-3, insulin and glucose concentrations did not predict mortality, after adjustment for age.

Predictors of mortality; gonadal and adrenal hormones

Serum luteinizing hormone, total and bioavailable testosterone, estradiol, estrone, DHEA, DHEAS and cortisol concentrations were not related to 4-year mortality after adjustment for age (Table 2). However, after adjustment for serum cortisol, subjects with DHEAS concentrations in the lowest quartile had a significant higher relative risk of mortality compared to subjects with DHEAS concentrations in the highest quartile (RR = 2.14 (1.04 – 4.41) for the 1st quartile versus RR = 1 for the 4th quartile, P=0.04). After adjustment for intima-media thickness of the bifurcation of the carotid artery, this relation was no longer significant (RR = 1.56 (0.75 – 3.25) for the 1st quartile versus RR = 1 for the 4th quartile, P=0.24).

Predictors of mortality; thyroidal hormones

After adjustment for age, high serum free thyroxine (T4) concentrations were significantly associated with an increased risk of 4-year mortality (Table 2). Serum triiodothyronine (T3) were not related to mortality. T3 was strongly related to albumin levels. However, after adjustment for albumin, high serum T3 levels were predictive of mortality (Table 2). Serum TSH, total T4, and reverse T3 were not related to 4-year mortality.

Table 1. Baseline characteristics of the study-population of 403 elderly men

General characteristics	**Mean (S.D.)**
Age (yr)	77.8 (3.6)
Number of diseases (0 - ≥5)	2.73 (1.72)
Smoking (number)	Ever: 351 Never: 51
Body Mass Index (kg/m2)	25.4 (3.04)
Intima-Media Thickness of carotid bifurcation (μm)	1.33 (0.56)
Number of plaques in carotid artery (n)	3.35 (2.64)
Physical characteristics	
Physical Performance (points)	8.5 (2.4)
Activities of daily living (points)	10.7 (4.3)
Maximum Leg Extensor Strength (Nm)	103.2 (20.9)
Isometric Grip Strength (kp)	34.3 (6.92)
Total Body Bone Mineral Density (g/cm^2)	1.17 (0.10)
Femoral Ward Bone Mineral Density (g/cm^2)	0.72 (0.16)
Somatotropic hormones	
Insulin-like Growth Factor-I (ng/l)	100.9 (29.2)
IGF- Binding Protein -1 (ng/l)	31.7 (15.5)
IGF- Binding Protein -2 (μg/l)	0.62 (0.32)
IGF- Binding Protein -3 (μg/l)	2.59 (0.70)
Insulin (mU/l)	8.91 (4.25)
Gonadal hormones	
Luteinizing hormone (U/l)	9.38 (8.41)
Total Testosterone (nmol/l)	8.83 (2.98)
Estradiol (nmol/l)	0.10 (0.06)
Estrone (nmol/l)	0.10 (0.04)
Adrenal hormones	
Dehydroepiandrostenedione (nmol/l)	7.34 (3.78)
Dehydroepiandrostenedione Sulfate (μmol/l)	1.96 (1.38)
Cortisol (nmol/l)	481.4 (183.9)
Thyroidal hormones	
Thyroid Stimulating Hormone (U/l)	1.15 (0.93)
Thyroxine (nmol/l)	80.4 (15.9)
Free Thyroxine (pmol/l)	16.6 (3.07)
Total Triiodothyronine (nmol/l)	1.43 (0.23)
Reverse Triiodothyronine (nmol/l)	0.33 (0.09)
Parameters of Inflammation	
Albumin (g/l)	45.6 (2.76)
C-Reactive Protein (mg/l)	3.87 (8.83)
Interleukin-6 (pg/ml)	191.7 (111.4)

Predictors of mortality; parameters of inflammation and nutrition

Low serum albumin concentrations were associated with a higher 4-year mortality rate (Table 2). Independent of age, baseline CRP levels predicted 4-year mortality (Table 2, Figure 2A). High serum interleukin-6 (IL-6) levels very strongly predicted 4-year mortality (Table 2). It appeared that only 1 subject had died amongst subjects with IL-6 concentrations in the lowest quartile (Figure 2B). Both CRP and IL-6 levels remained predictive of mortality after adjustment of parameters of subclinical atherosclerosis.

Relations between the predictors of mortality

To determine the relative importance of these predictors, we performed a multivariate analysis, including all parameters which were significantly related to 4-year mortality. The result of this analysis is shown in Table 3. In this multivariate analysis, the intima-media thickness of the carotid bifurcation was not included, because due to logistic reasons the measurement was not performed with 107 subjects. However, if the intima-media thickness was included, the results were comparable to the results described in Table3. The significant parameters of the model described in Table 3 results in an area under the Receiver Operating Curve characteristic of 0.78, indicating that with these parameters the prediction was correct in 78% of the subjects whether or not they would die within 4 years (Table 4).

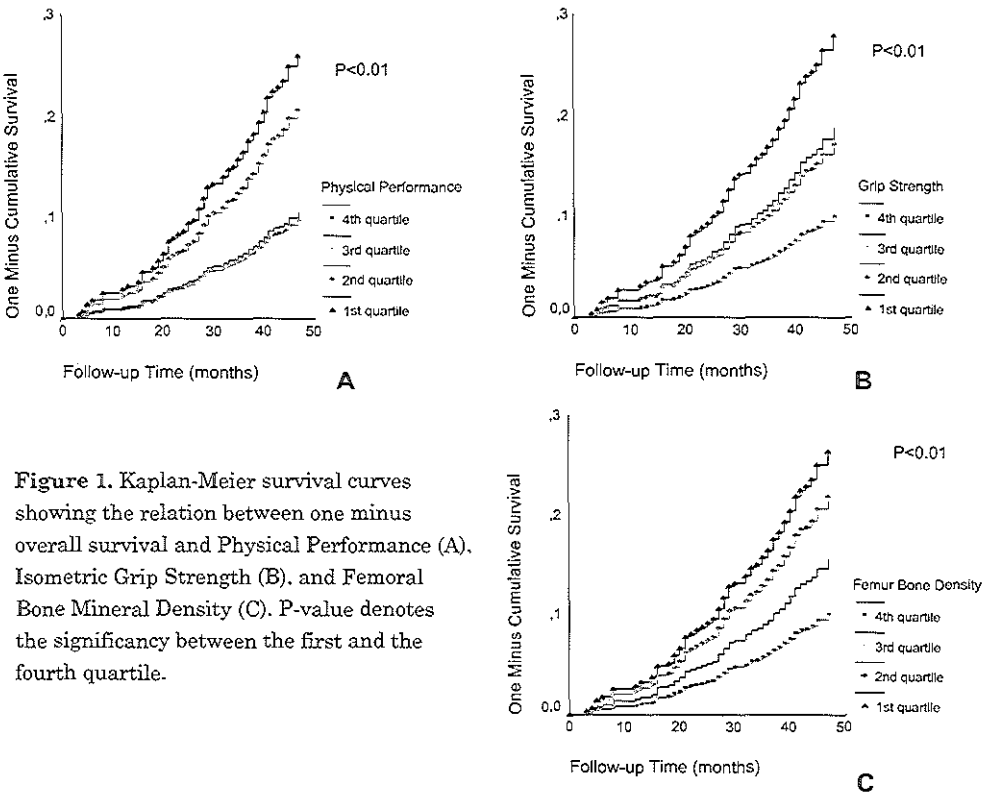

Figure 1. Kaplan-Meier survival curves showing the relation between one minus overall survival and Physical Performance (A), Isometric Grip Strength (B), and Femoral Bone Mineral Density (C). P-value denotes the significancy between the first and the fourth quartile.

Table 2. Predictors of 4-year mortality.

General characteristics	**Relative Risk (95% CI)**	**P-value**
Age (yr)	1.39 (1.14 – 1.70)	0.001
Number of diseases (0 - ≥5)	1.50 (1.03 – 2.17)	0.04
Smoking (current + ever vs never)	2.61 (1.04 – 6.56)	0.04
Body Mass Index (kg/m2)	0.91 (0.71 – 1.15)	0.45
Intima-Media Thickness of carotid bifurcation (μm)	1.35 (1.06 – 1.72)	0.01
Number of plaques in carotid artery (n)	1.27 (1.02 – 1.57)	0.03
Physical characteristics		
Physical Performance (points)	0.71 (0.57 – 0.89)	0.004
Activities of daily living (points)	1.19 (1.00 – 1.43)	0.06
Maximum Leg Extensor Strength (Nm)	0.75 (0.60 – 0.95)	0.02
Isometric Grip Strength (kp)	0.70 (0.55 – 0.90)	0.005
Total Body Bone Mineral Density (g/cm^2)	0.81 (0.64 – 1.01)	0.07
Femoral Ward Bone Mineral Density (g/cm^2)	0.74 (0.57 – 0.95)	0.02
Somatotropic hormones		
Insulin-like Growth Factor-I (ng/l)	0.95 (0.75 – 1.21)	0.68
IGF- Binding Protein -1 (ng/l)	1.29 (1.06 – 1.58)	0.01
IGF- Binding Protein -2 (μg/l)	1.22 (1.01 – 1.48)	0.04
IGF- Binding Protein -3 (μg/l)	0.90 (0.69 – 1.17)	0.44
Insulin (mU/l)	1.09 (0.89 – 1.35)	0.40
Gonadal hormones		
Luteinizing hormone (U/l)	0.94 (0.75 – 1.18)	0.60
Total Testosterone (nmol/l)	0.92 (0.74 – 1.14)	0.45
Estradiol (nmol/l)	0.87 (0.66 – 1.14)	0.32
Estrone (nmol/l)	1.01 (0.78 – 1.31)	0.92
Adrenal hormones		
DHEA (nmol/l)	1.07 (0.87 – 1.31)	0.51
DHEAS (μmol/l)	0.84 (0.64 – 1.10)	0.20
Cortisol (nmol/l)	1.19 (0.95 – 1.48)	0.12
Thyroidal hormones		
Thyroid Stimulating Hormone (U/l)	1.05 (0.85 –1.30)	0.62
Thyroxine (nmol/l)	1.22 (0.96 – 1.55)	0.11
Free Thyroxine (pmol/l)	1.27 (1.01 – 1.60)	0.04
Total Triiodothyronine (nmol/l)	1.34 (0.89 – 1.44)	0.30
Total Triiodothyronine adjusted for albumin	1.30 (1.02 – 1.66)	0.03
Reverse Triiodothyronine (nmol/l)	1.14 (0.91 – 1.43)	0.25
Parameters of Inflammation		
Albumin (g/l)	0.68 (0.53 – 0.86)	0.002
C-Reactive Protein (mg/l)	1.33 (1.07 – 1.67)	0.01*
Interleukin-6 (pg/ml)	1.74 (1.35 – 2.24)	<0.001*

The Relative Risk and it 95% CI was based on the hazard ratio per standard deviation increase.
* C-reactive Protein and Interleukin-6 levels were logarithmically transformed

Table 3. Relative Risk of 4-year mortality after performing a multivariate analysis including the significant (P<0.05) predictors of mortality of Table 2.

Mortality	Relative Risk (95% CI)	P-value
Age (year)	1.47 (1.09 – 1.98)	0.01
Number of diseases	1.31 (0.78 – 2.20)	0.31
Smoking (current +ever vs never)	2.83 (0.88 – 9.10)	0.08
Number of plaques (n)	1.19 (0.89 – 1.58)	0.23
Physical Performance (points)	0.90 (0.64 – 1.26)	0.53
Maximum Leg Extensor Strength (Nm)	0.95 (0.65 – 1.39)	0.80
Isometric Grip Strength (kp)	0.83 (0.58 – 1.18)	0.30
Femoral Ward Bone Mineral Density (g/cm^2)	0.70 (0.53 – 0.94)	0.02
Insulin-like Growth Factor Binding Protein-1 (ng/l)	0.75 (0.52 – 1.09)	0.13
Insulin-like Growth Factor Binding Protein-2 (µg/l)	0.87 (0.58 – 1.28)	0.47
Free T4 (pmol/l)	1.38 (1.03 – 1.85)	0.03
T3 (nmol/l)	1.70 (1.18 – 2.45)	0.004
Albumin (g/l)	0.54 (0.38 – 0.77)	0.001
C-Reactive Protein (mg/l)	1.51 (1.14 – 2.01)	0.004*
Interleukin –6 (pg/ml)	1.86 (1.30 – 2.68)	0.001*

The Relative Risk and its 95% CI was based on the hazard ratio per standard deviation increase.
* C-reactive Protein and Interleukin-6 levels were logarithmically transformed

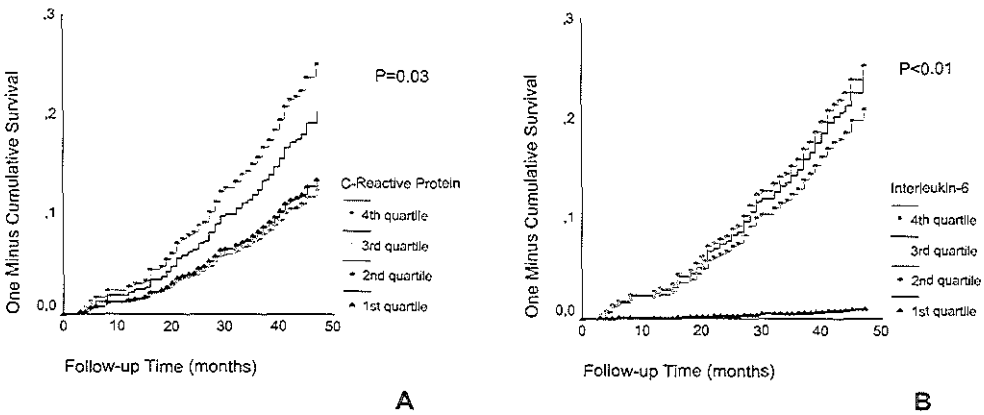

Figure 2. Kaplan-Meier survival curves showing the relation between one minus overall survival and C-Reactive Protein (A) and interleukin-6 concentrations (B). P-value denotes the significancy between the first and the fourth quartile.

Table 4. Additional values of the strongest predictors of 4-year mortality in a ROC analysis model.

Mortality	ROC %
Age (year)	61
Femoral Ward Bone Mineral Density (g/cm²)	68 (+7)
Albumin (g/l)	70 (+2)
Free T4 (pmol/l)	71 (+1)
T3 (nmol/l)	71 (+0)
C-Reactive Protein (mg/l)	73 (+2)
Interleukin –6 (pg/ml)	78 (+5)

The area under the Receiver Operating Curve (ROC) characteristic of 0.50 is the starting point, indicating that without being informed of any parameter, the prediction will be correct in 50% of the subjects whether or not they will die within 4 years. With age alone the prediction was correct in 61%. With age plus bone mineral density the prediction was correct in 68%. With all the parameters in this table the prediction was correct in 78% of the subjects whether or not they would die within 4 years.

DISCUSSION

To the best of our knowledge, this is the first study in which apart from the well-known conditions like smoking, co-morbidity and intima-media thickness of the carotid artery, the relative importance of a range of possible physical, hormonal and inflammatory predictors of mortality was investigated in a single group of 403 non-disabled, independently living elderly men. With regard to physical functional ability, low physical performance, muscle strength and bone mineral density all predicted 4-year mortality. Of the endocrine system, high IGFBP-1, IGFBP-2, T3 and free T4 concentrations were all predictive of a higher mortality risk. Finally, low serum albumin and high C-reactive protein and interleukin-6 levels were associated with a higher incidence of 4-year mortality, while never having smoked predicted survival. With the seven most significant parameters 78% of subjects could be correctly identified with respect to alive or death after four years (area under the ROC curve of 0.78).

To appreciate the findings of this study, some of its characteristics should be addressed. Only independently living men who were able to visit the study center by themselves were included. Also cognitive function of these individuals was largely intact. Only 30 individuals scored below 24 points in the mini-mental state examination, indicating mild cognitive impairment. The findings therefore pertain to relatively healthy, active old men, and may not necessarily apply to the whole population of elderly men, many of which live in dependent, supervised living conditions or in nursing homes. However, in elderly men with a broader range of functional ability, associations might well have been stronger.

Results from recent studies suggest that physical performance in older adults inversely predicts mortality independent of disease (24-26). This is confirmed in our population of elderly men. In addition, both lower muscle strength, mainly isometric grip strength, as well as lower bone mineral density were significantly associated with mortality independent of disease. Rantanen et al. recently described in a much younger population that hand grip strength is a long-term predictor of mortality in initially healthy men (27). Johansson et al. as well as Trivedi and Khaw recently described that low bone mineral density, apart from disease, is also a predictor of mortality in the elderly (28,29). Muscle strength and bone mineral density may be markers for general health or lead to imbalance and an increase in fractures. Both notions are associated with an increased mortality.

Part of the age-related physiological changes might be influenced by the activity of the endocrine system (30,31). Therefore one could hypothesize that serum hormone concentrations not only relate to measures of functional ability, but also predict mortality. High IGFBP-1 and IGFBP-2 concentrations were both associated with an increased risk of death. We previously described that high IGFBP-1 levels are associated with measures of atherosclerosis (32). After adjustment for the presence of cardiovascular disease in particular, mean intima-media thickness of the carotid bifurcation, IGFBP-1 no longer predicted mortality, suggesting that increasing IGFBP-1 levels reflect the presence of (sub)clinical atherosclerosis. Further, since serum IGFBP-1 levels seem to mirror insulin secretion (33), they may serve as an indicator of insulin resistance.

In this population serum IGFBP-2 levels were strongly related to all measures of functional ability (data not shown), as well as mortality. In the multivariate analysis IGFBP-2 lost its significant association with mortality, suggesting that this relation may largely act through functional ability.

Although testosterone was strongly related to bone mineral density and muscle strength in this study (13), testosterone was not independently related to mortality. Most studies describe that testosterone substitution in elderly men cause an increase in lean body mass, muscle strength and bone density (7). Testosterone might therefore influence mortality indirectly by improving functional ability via muscle strength and bone density.

Subjects with the lowest DHEAS levels had a significantly higher risk of mortality compared to subjects with DHEAS levels in the highest quartile. These findings are in agreement with previous studies (29,34), in which it was also found that in men with DHEAS levels in the lowest quartiles, mortality rates are the highest compared to subjects with higher DHEAS levels. In our study, this difference was no longer significant after adjustment for subclinical atherosclerosis. It has been hypothesized that DHEAS levels might be protective for cardiovascular disease. However, the clinical significance of DHEA in cardiovascular disease remains uncertain (35). Low DHEAS levels, on the other hand, might also be an epiphenomenon related to the presence of disease (36).

Recently, it has been described that in a population-based study, subclinical hyperthyroidism predicts mortality (37). We could not confirm these results. Only a few subjects satisfied the criteria for subclinical hyperthyroidism (low TSH and normal free T4). However, we found that subjects with T3 levels in the lowest quartile, which were beneath the normal values for healthy adults (<1.42 mmol/l), had a significantly better 4-year survival compared the other subjects. These subjects had normal reverse T3 and TSH levels. It can be hypothesized that the age-related decline in T3 levels might serve as an adaptive mechanism, analogous to the euthyroid sick syndrome during illness, to prevent excessive catabolism and consequently may influence mortality.

In addition, higher free T4 levels, within the normal range, were associated with a higher risk of mortality. This relation appeared to be very strong and independent of e.g. disease, bone density and specific medication known to influence thyroid function. Although no definitive conclusions can be made, it should be mentioned that we were not informed about the possible subsequent development of overt hyperthyroidism in the population studied. Also, no information about autoimmune antibodies was available. Although serum reverse T3 concentrations were strongly inversely related to parameters of physical ability (data not shown), they did not predict mortality. This might indicate that increased reverse T3 concentrations are a sign of an adaptive mechanism to prevent excessive catabolism and therefore are not related to mortality.

It has been established previously that low albumin levels are an independent predictor of mortality in different populations (reviewed by Goldwasser et al. (38)). In the multivariate analysis, the albumin concentration was one of the strongest predictors of mortality, indicating a very strong representation of an individuals' condition. Low albumin levels may reflect an impaired nutritional status and/or the presence of an acute phase reaction, as albumin is a negative acute phase protein.

It has been demonstrated before that C-reactive protein (CRP) and interleukin (IL)-6 concentrations reflect inflammation and predict cardiovascular disease, disability as well as mortality in a healthy, non-disabled population with or without cardiovascular disease (39,40). In this population CRP and IL-6 levels, within the normal range, both strongly predicted mortality. Remarkably, these associations were independent of co-morbidity, subclinical cardiovascular disease, cardiovascular risk factors, the use of non-steroidal anti-inflammatory drugs and physical ability. IL-6 is a cytokine involved in the regulation of the acute phase response and bone metabolism. IL-6 decreases the production of albumin by the liver and is known to influence multiple endocrine axes (41,42). According to Figure 2, it is perhaps more appropriate to conclude, that low levels of IL-6 predict survival. This is in contrast to a study by Harris et al. (39), reporting that subjects with IL-6 levels in the highest quartile have a higher risk of mortality compared to those with lower IL-6 levels. Low IL-6 and CRP levels in the normal range may therefore be indicators for the absence of (subclinical) disease in general in the very old.

Physical functional ability, hormonal and inflammatory parameters appear to be well informative to establish the mortality risk. It is remarkable that in this relatively healthy population, several physical, hormonal and inflammatory parameters are strongly related to mortality, independent of co-morbidity and atherosclerosis. It should

be noted that most concentrations are within the normal range. In this study we focused on the relative importance of these predictors in order to establish prognosis. The independent value of the individual predictors of mortality, needs to be further examined. This may have important implications for future intervention strategies, notably exercise programs, hormonal or anti-inflammatory treatment.

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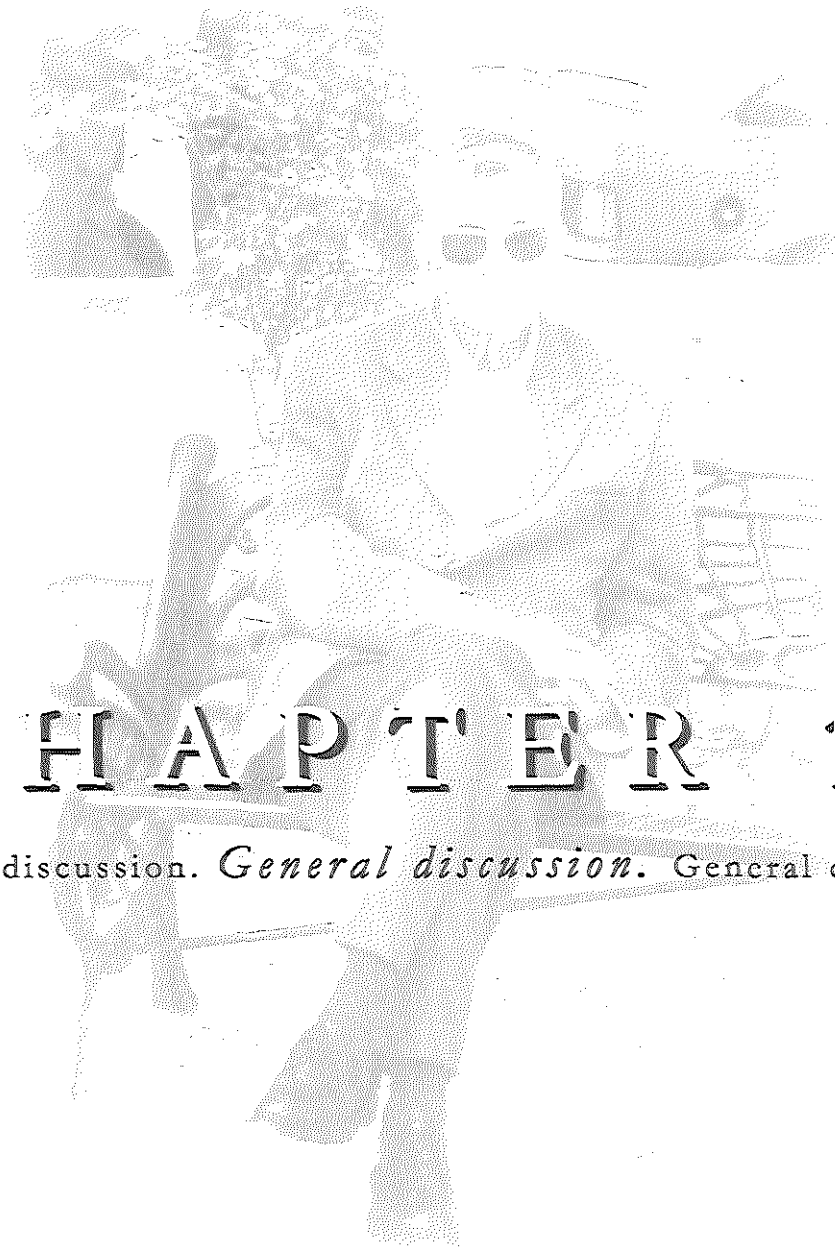


CHAPTER 10

. General discussion. *General discussion.* General discussion.

GENERAL DISCUSSION

1. AIM OF THE THESIS
2. STUDY POPULATION
3. STUDY METHODS
    - A. Physical functional ability
    - B. Body Composition
    - C. Quality of life, loneliness and cognitive function
    - D. Ultrasound of the carotid artery
    - E. Serum hormone concentrations and parameters of inflammation
4. RESULTS
    - A. Physical functional ability and Quality of life
        - *a) Determinants of functional ability*
        - *b) How to define chronological and successful aging*
            1. *Chronological age*
            2. *Successful aging*
    - B. Endocrine aspects of aging
        - *a) Somatotropic axis*
        - *b) Pituitary-Gonadal axis*
        - *c) Pituitary-Adrenal axis*
        - *d) Pituitary-Thyroidal axis*
    - C. Co-morbidity, atherosclerosis and inflammation
5. CONCLUSIONS

1. AIM OF THE THESIS

The aim of this thesis was to obtain more insight in the relations between the endocrine system and the physical and psychological aging process in elderly men. A number of questions were addressed.

1) Since it is difficult to define those subjects that age successfully with respect to physical function, we set out to determine which are the main physiological determinants of functional ability in the elderly. We also investigated which of the physical parameters predicted mortality. To emphasize the importance of the parameters of physical function, we assessed their relationship with quality of life.

2) As the age-related decline in serum hormone levels occurs in parallel with the loss of physical function, bone mass, muscle mass and muscle strength, we investigated whether serum hormone levels of all endocrine axes involved were related to the physical functions measured, including bone mineral density and body composition. In addition, we defined whether these hormone concentrations could be used as an indicator of a good physical functional ability as well as survival or mortality. Furthermore, we established whether serum hormone concentrations were related to quality of life.

3) Because serum sex hormones are mainly bound to their hormone binding globulins and albumin, it remained to be established whether the bioavailable hormone fraction is the best representation of the bioactive hormone concentration. Therefore, various measures were compared of bioactive androgens and estrogens to which tissues are exposed in their relationship with muscle strength, bone density and body composition.

4) Because a common variant form of luteinizing hormone (LH) was detected in apparently healthy individuals, caused by point mutation-based substitutions of two amino acids (Trp8Arg and Ile15Thr) in the LHβ subunit (1-3) and since it was suggested that the in vivo bioactivity of the LH variant is lower than that of the wild-type hormone (4,5), we determined whether differences in bioactivity of the variant LH were related to characteristics of physical functional ability. In addition the presence and concentration of this genetic LH variant in elderly men was determined. Also, its potential value as an 'integrated' marker of both androgen and estrogen bio-activity was considered.

5) Since atherosclerosis is an important cause of cardiovascular morbidity and mortality during aging, we assessed the presence of atherosclerosis by measurement of the carotid artery intima-media thickness and the number of plaques. In addition, as changes in endocrine systems may influence the atherosclerotic process, e.g. a decreased activity of the GH – IGF-1 axis, the relationships between plasma hormone concentrations and parameters of atherosclerosis were determined.

6) It has been demonstrated before that C-reactive protein (CRP) and interleukin-6 (IL-6) concentrations reflect inflammation and predict cardiovascular disease as well as mortality in a healthy, non-disabled population with or without cardiovascular disease (6,7). Also in our population it was investigated whether CRP and IL-6 were related to mortality.

.7) Finally, an attempt will be made to integrate the results of this thesis in relation to the concept of successful aging. The definition of successful aging as proposed by Rowe was taken as a starting point.

2. STUDY POPULATION

Names and addresses of all male inhabitants 70 years and older were drawn from the municipal register of Zoetermeer. The study was intended to include 400 subjects. Finally, 1567 men were invited. A total of 886 men did not respond to the mailed invitation in which it was mentioned that only subjects who lived independently and had no severe mobility problems could participate. A third requirement was that the participants did not have signs of dementia. Response rate of subjects invited aged □ 80 yr was 18.3% compared to 32.7% of subjects aged < 80 yr. After exclusion of subjects who did not live independently and of subjects with signs of dementia (measured by the mini-mental state examination), 403 men participated (25.7%).

After taking a medical history and performing a complete physical investigation 5 groups of co-morbidity were established as described in chapter 9 (those with none, one, two, three, four or five or more diseases or complaints). In addition, two groups were formed using medication or none at all. None of the subjects were treated for systemic infections or malignancies at the time of inclusion in the study.

Several aspects need to be discussed with regard to the study population and the way of inclusion.

Aging can be approached in two different ways: one can direct one's attention to the ensuing deficits, or to the factors which play a protective role to the decline in function. These different approaches which are reflected in the concepts 'frailty' and 'successful aging' need to be explained. Frailty is defined as a syndrome of multi-system reduction in physiological capacity as a result of which an older person's function may be severely compromised by minor environmental challenges, giving rise to the condition 'unstable disability' (8). The variable presence of co-morbidity makes research findings more difficult to generalize. Therefore the alternative to focus research on the least frail and 'non-diseased', which implies the successfully aged, might be easier. Older persons with minimal physiologic loss, or none at all, when compared to the average of their younger counterparts, can be regarded as having aged more broadly successful in physiologic terms (9). The concept frailty focuses mainly on the physical aspects of aging, while the concept of successful aging includes a broader range of aspects such as physical, psychological and social aspects. Both concepts are not easy to define in a single measure, nor are there generally accepted criteria to categorize a certain individual.

Although definitions of successful aging in gerontology are numerous, there is still no consensus on the definition of successful aging. Rowe et al defined it as including three main components: low probability of disease and disease-related disability, high cognitive and physical functional capacity, and active engagement with life (Figure 1) (10). Fries et al. defined successful aging as optimizing life expectancy, while simultane-

ously minimizing physical, psychological and social morbidity (11). Vaillant argued that in addition to physical health, there are three further dimensions, or outcomes, of successful aging: mental health, psychosocial efficiency and life satisfaction (12).

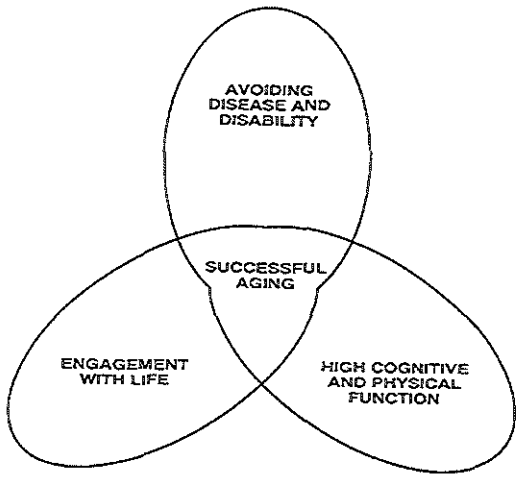


Figure1. The concept of 'Successful Aging' as illustrated by the model by Rowe with 'maintaining high cognitive and physical functional capacity', 'avoiding disease and disability', and 'maintaining active engagement with life' as main determinants.

In our study we could have approached aging by investigating frailty, which is defined as 'a state of reduced physiological reserves associated with an increased susceptibility to disability' (13).

In our study population we focused on the least frail, which implies the successfully aged. To decrease the number of subjects with severe diseases to a minimum, we invited subjects who lived independently, had no severe mobility problems and did not have signs or symptoms of dementia. We acknowledge that we were not able to determine all aspects which compromise or influence successful aging as described by Rowe and illustrated in Figure 1. We did not investigate a random selection of the 80 year old male in The Netherlands, and therefore we cannot generalize the prevalence of co-morbidity and mean level of physical functioning to the general population. This was not a disadvantage in our study, since the aim was not to measure frequency, but to assess associations. In fact, in less selected populations with a broader range of functional ability, the associations found between parameters of the endocrine system and physical and psychological well-being are likely to be stronger.

As mentioned above, it was not possible to completely describe or define successful aging. As will be explained in the following section, we used several questionnaires

representing aspects of quality of life as endpoints. Since also these quality of life questionnaires do not fully describe successful aging, we used mortality data as a derivative as well. Four years after the initial investigation 75 of the initial 403 men had died. This number was obtained from the municipal register of Zoetermeer. Since information concerning the cause of death could not be provided, the general practitioners were notified. The general practitioners returned the forms of 56 subjects. A cause of death was given of 49 of these men, while of 7 men the cause was unknown. The main reason not to fill in the mortality form of the other 19 subjects, was a lack of cooperation or a loss of follow-up of the participants. The causes of death were in almost all cases defined by the attending general practitioners, as the great majority of these men had died at home. Although most individuals were given a diagnosis at death (mostly ischemic heart disease, pneumonia, consequences of a fall, or cancer), one cannot rely very much on these diagnoses, presented on the death certificates. Most very old individuals die with a variety of diagnoses, and the real cause of death remains uncertain. A review of autopsy findings in 200 persons older than 85 years identified no acceptable cause of death in at least 30% of the cases (14).

3. STUDY METHODS

A. Physical functional ability

1. Subjective functional ability was assessed using the ***Modified Health Assessment Questionnaire*** (15). Several instruments for the functional assessment of older subjects have been developed. Self-administered or interviewer-administered instruments subjectively judge a persons' ability. Although the ability to perform activities of daily living (ADL) has at times been synonym for physical function, ADL scales actually assess the basic capacity of persons to care for themselves and hence represent a narrow range of performance (16,17). Our population is probably a relatively strong and healthy sample from the general population. In addition, one of the inclusion criteria stated that subjects needed to be living independently. Variability of the scores of the modified health assessment questionnaire in our population was skewed, with a relatively large percentage of subjects scoring in the lower range of this questionnaire (having few problems in ADL). These factors suggest that it might have been more useful to utilize an ADL scale to assess somewhat higher levels of performance, like instrumental ADL scales (18). Scales that assess a wider range of performance can more accurately classify the level and range of disability in any group of patients and are more likely to detect clinically important changes. Despite the small variability in ADL in our study group, we found several strong relations between different parameters of physical activity and ADL, suggesting that a greater variability when using a questionnaire with a wider range of performance would have resulted in even stronger associations.

2. Objective functional ability

We were initially concerned that self-reported function would provide insufficient discriminatory power to describe the variation in impairments. Therefore, also a physical functional performance test was used (19). Relative to questionnaires, performance measures were not found to be more acceptable to respondents, easier to administer, or easier to interpret by several authors (20). Self-report and objective performance measures complement one another, and add both to the understanding of older person's functional status (21,22). The ***physical performance test developed by Guralnik et al.*** used in this study has proven to be valid and easy to administer (21). This test does not require special equipment and can be performed in a few minutes. The combinations of the three parts of the test seem to give a good representation of physical function. It has been demonstrated previously several times that this performance test predicts the onset of disability in those initially reporting no disability in activities of daily living (23).

Physical performance tests are better than self-reported disability in measuring changes during treatment / intervention, because small changes are easy to detect. ADL is an end-point for the weakest, and minor decreases in physical performance do not have to result in difficulties in ADL. Scores of ADL and the physical performance test (PPS) in our population were strongly related. Most of the associations between hormone measurements or physical characteristics and ADL were also present with PPS. However, some hormones were only related to ADL and other hormones were only related to PPS (next chapter). It therefore remains unanswered what the additional clinical or research benefits are of combining scores produced by different methods or items from different instruments in order to obtain the most accurate assessment of physical function.

Muscle strength is also a measure of physical functional ability. It is well-documented that the reductions in strength that occur with advancing age have negative consequences on gait and other aspects of physical performance capacity, particularly if the age-related decline in strength is exaggerated by inactivity or debilitating disease (13,24,25). From a physiologic and kinesiologic point of view, the measurement of strength is very complex. The force produced by a muscle group is influenced by the speed of limb movement and the mode of muscle contraction – isometric (static), eccentric (lengthening) and concentric (shortening). The muscle groups chosen to represent strength should be based on the relevance to the task of interest (26). Two types of strength measurements were performed in this study.

Isometric grip strength in kilo pounds (IGS) was tested using an adjustable hand held dynamometer (JAMAR dynamometer) in the non-dominant hand (27). Each test was repeated three times and the average was used in the analysis.

Isometric leg extensor strength was measured as described by Hsieh and Philips using the Hoggan MicroFET hand held dynamometer (28). The measurements were done as described in chapter 3. Few studies so far have described leg extensor strength (LES) measured with the Hoggan microFET dynamometer (29). Kwoh et al. studied the reliability of the MicroFET in subjects after elective hip and knee athroplasty (30). He concluded that inter- and intra-assay variability came up to meet the requirements. According to Hsieh et al, manual dynamometry is an acceptable method when the "break" test is used (28). Judgment of the strength using this method is dependent on the interpretation of the investigator. Since in the cross-sectional part of this study, two investigators measured LES, it was checked regularly during the course of the investigation whether the two investigators performed the strength measurements in a similar way, with the same results. A reliability investigation recommended that measurements of leg extensor strength with the microFET should be performed by a maximum of two persons to meet up the requirements of the inter-observer variability (31). In our study, a few subjects (n=12) with isometric grip strength between 30 and 50 kg reached the maximum achievable value of the microFET, as shown in Figure 2. Because of the small number this had no implications for our analyses. However, this technique may not be suitable for younger and more powerful subjects. Bohannon et al. indeed reported that weaker subjects gave higher reliability of muscle strength measurements than stronger subjects (32,33). When the leg was held at 120°, LES was significantly higher compared to the extended position. This is to be expected, as the biomechanical circumstances for the quadriceps are more favourable at 120°. In addition, the insertion distance to the turning point is larger at 120° than at 180°. Moreover, when the quadriceps is more extended at 120°, force per moment increases. Although the non-dominant leg is in general slightly weaker, there was almost no difference in the relation between strength of the dominant and the non-dominant leg and other variables.

The marked associations between the measurements in different positions, and between isometric grip strength (IGS) and all the measurements of LES (Figure 2), indicate that measuring knee extensor strength at 120° with the Hoggan microFET dynamometer provides an easy method to measure strength in elderly men, especially in a home-based setting. This is also illustrated by the normal distribution in our population (Figure 3).

B. Body Composition

Bone mineral density was measured using dual energy X-ray absorptiometry. This is a standardized method to measure bone density and equilibration was performed routinely every morning. We measured bone mineral density of total body and femur (neck, ward and trochanter). We did not measure bone mineral density of the lumbar spine, since in this older population the results of such a measurement will be disturbed by the presence of arthrosis.

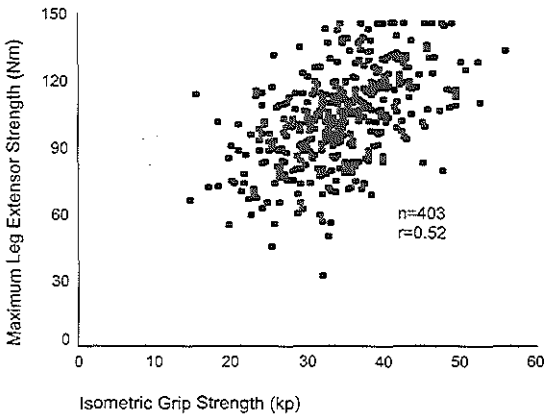


Figure 2. Association between Isometric Grip Strength and Leg Extensor Strength.

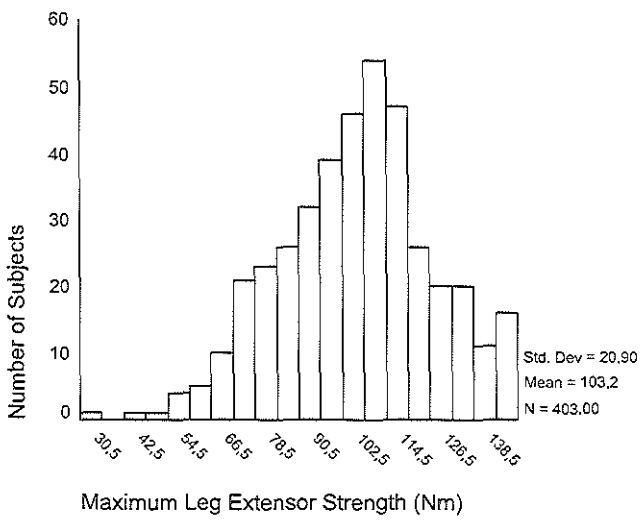


Figure 3. Distribution of Leg Extensor Strength in 403 elderly men.

Fat mass and lean body mass were also measured using dual energy X-ray absorptiometry. It is not possible to differentiate between subcutaneous and visceral fat mass with this technique. Because it is mainly the visceral fat we are interested in, some relations may not precisely describe the exact associations. This has to be taken into account when interpreting the results.

Neither is it possible to differentiate between skeletal muscle mass and smooth muscle mass. However, most of the differences in muscle mass between the participants will be in skeletal muscle, and therefore it can be used to describe its relations with for example hormone levels. The strong positive relation between leg extensor strength and lean body mass, suggests that it is indeed a good method in measuring lean body mass.

C. Quality of life, loneliness and cognitive function

Successful aging compromises terms like psychological well being, ***quality of life (QoL)*** and life satisfaction, which are all used interchangeable. Although there are no generally accepted definitions and approaches for assessment, there is broad agreement that in measuring health-related quality of life we should assess a number of crucial areas including physical function, psychological state, somatic symptoms, social function including relationships, sexual function and occupational function and possibly financial state (34). With reference to this agreement, the terms successful aging and QoL can also be used interchangeably. However, QoL is measured by subjective indicators only, while successful aging can be measured by both objective and subjective indicators. There are numerous general and ill-specific quality of life (QoL) questionnaires, which are critically reviewed by Gill and Feinstein (35). Of main importance is that there are marked inter- and intra-individual differences in the perception and evaluation of objective aspects of life or disease. For example, the "illness paradox" describes the discrepancy between "objectively" poor health (as assessed by an outside observer) and a positive self-assessment. In this study we used a QoL questionnaire (QLS), described in chapter 7, which has been recently developed by Henrich and Herschbach (36). This is a questionnaire, in which in its development the attempt was made to deal adequately with the problem of the relative importance of individual aspects of quality of life. Recent studies with QLS have shown that weighing of the individual items for their importance to the respondent is an effective way to incorporate the concept of subjectivity of quality of life in the quality of life instrument (36). This questionnaire has been validated for the Dutch population (36). In our population the questionnaire was relatively easy to administer after a good explanation. It took less than 30 minutes to fill in the three modules of the questionnaire, which is important against the potentially decreasing attention span of the oldest old.

During the follow-up study, 4 years after the initial investigation, health-related quality of life was also measured using the EuroQol EQ-5D instrument, a widely used and validated generic quality of life instrument with 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has three levels: no limitations, some limitations and severe limitations. In addition, the participants were asked to describe their overall actual health state using the EuroQol visual analogue scale (VAS), where 0 represents the worst imaginable health state and 1 indicates perfect health.

Loneliness was measured using the Loneliness Questionnaire where possible scores range from 0 (not lonely) to 11 (extremely lonely) (37). This questionnaire has previously been shown to be reliable and valid (37,38).

The Mini-Mental State Examination was used to assess cognitive function. Only 30 subjects had a score between 18 and 24 points, which indicates mild cognitive impairment. The other subjects scored above 24 points. Most of the subjects scored only 2 questions wrong, and therefore this test was not suitable to differentiate between subtle cognitive distinctions (39).

D. Ultrasound of the carotid artery

As a measure of generalized atherosclerosis ultrasound of the internal carotid artery was performed. The intima-media thickness of the common carotid artery and the bifurcation was measured as well as the number of plaques (described in chapter 6). Thickening of the intima-media of the carotid artery is generally considered to be a sensitive early marker of generalized atherosclerosis since such thickening has been associated with an unfavorable cardiovascular risk profile (40-42), other localizations of atherosclerosis (43,44) and an increased risk of myocardial infarction (45,46).

E. Serum hormone concentrations and parameters of inflammation

In this study serum concentrations of hormones and hormone binding proteins of the growth hormone, gonadal, adrenal and thyroid axes were measured as described in the previous chapters. To minimize the diurnal as well as the seasonal variation all blood samples were obtained in the summer (May until beginning of September) in a fasting condition (between 8.00 and 10.00 hr a.m.) and stored at -40°C. The storage time varied from 1 to 5 months.

Serum ***IGF-I*** and ***IGFBP-3*** levels show little circadian fluctuations (47). Serum ***IGFBP-2*** levels slightly increase in the evening (48). The main regulator of serum ***IGFBP-1*** levels is ***insulin***, which negatively influences IGFBP-1. To obtain standardized measurements of glucose, insulin, IGFBP-1 and BP-2 concentrations all blood samples were taken in a fasting condition.

Testosterone concentrations in men have a slight diurnal rhythm, which decreases during aging, being the lowest in the morning (49). Serum testosterone concentrations in our population were relatively low compared to studies, which have been described previously in somewhat younger populations (50). First of all there are no reference values for testosterone concentrations in older men. Mean serum testosterone concentrations have never been described in a relatively large population of this age group. The relatively low testosterone concentrations might also be due to different methods used to measure testosterone concentrations. Results in different tests are especially variable at low concentrations (51). Before trying to compare testosterone concentrations among older populations, reference standards are needed. And even then it is difficult to compare cohorts, since testosterone concentrations are probably also related to the presence of co-morbidity.

With regard to ***estradiol*** and ***estrone*** concentrations in elderly men, little is known. No reference values are available for these measurements either. Estradiol and estrone seem to be relatively stable during the day (49). To minimize the variation all samples

were measured in one single assay. Serum estradiol concentrations were normally distributed in our population.

Plasma ***DHEAS*** levels reflect synthesis by the adrenal cortex, because the adrenal is normally the predominant source of DHEAS. Unconjugated ***DHEA*** is present in much lower circulating levels. Unconjugated DHEA and cortisol share a common circadian rhythm (52). A low metabolic clearance rate for DHEAS results in uniformly high plasma and therefore stable DHEAS levels.

In contrast to serum ***T3, T4 and reverse T3***, serum ***TSH*** shows a circadian rhythm, with a nocturnal surge.

It was not possible to measure the ***T4 sulfate (T4S)*** concentrations of all samples in a single assay. Intra- and inter-variability coefficients of the T4S assay were 17% and 19%, respectively, in the low concentration range measured in these elderly men. Although T4S concentrations were normally distributed in our population, the results presented in chapter 5 should be interpreted with caution, because the T4S concentrations were rather low in this population and as a consequence the assay quality is also low.

C-Reactive Protein (CRP) concentrations were determined by a high sensitive method using a latex-enhanced immunoephelometric assay on a BN II analyser.

Interleukin-6 was measured using the commercially available Immulite assay. It is unclear whether interleukin-6 has a circadian pattern in the circulation (53,54). However, in one study, the intraclass correlation coefficient was 0.87 for plasma interleukin-6 levels that were measured eight times during 36 days, suggesting stability for at least one month (55).

4. RESULTS

Before we discuss the results of this thesis, some aspects of cross-sectional studies need to be addressed. A particular difficulty of cross-sectional studies looking at associations concerns the cause and consequence of the measurement of interest and the possible risk factor. For example, in the study of the relations with quality of life, we found that subjects with the lowest quality of life have the lowest muscle strength. We might conclude from this that low muscle strength leads to a lower quality of life, but an equally valid possibility is that low quality of life leads to reduced muscle strength. Because we have collected both sets of information at the same time we cannot draw a clear inference of causality.

It has to be mentioned that if two laboratory values are both related to the dependent variable, adjusting for one another to figure out which is the more potent indicator is difficult, because the direction of cause and consequence are unknown. In addition, when two parameters are strongly related it might be possible that both parameters loose their significance with the dependent variable when adjusted for one another. For example, as described in chapter 6, serum testosterone and estrone concentrations were both related to intima-media thickness. It may be that only one of these hormones is related to

intima-media thickness and that the other one is just a confounder, since testosterone and estrone by themselves are strongly interrelated. By performing this adjustment we were not able to determine which of these hormones was most powerfully related to intima-media thickness. In contrast, when mutually adjusted both hormones lost the significance of their relationship with intima-media thickness. However, one cannot conclude that the relations between testosterone and estrone with intima-media thickness have no significance.

It is important not only to know whether a relation is statistically significant, but it is also important to be informed about the strength of the significant relations. The linear regression coefficient, which is the slope of the equation, describes the degree of change which is necessary to in- or decrease the dependent value with one unit. So, to determine whether a relation is biologically and/or clinically significant, one should examine the regression coefficient. Another statistic by which we can be informed about the strength of the association, although somewhat less intelligible, is the R-squared value (R^2) which represents the proportion of the total variation explained by the model. For example, the significant association between quality of life (of the health part) and leg extensor strength has a P-value of <0.001 and a R^2 of 0.11, which implies that 11% of the variation in quality of life is explained by muscle strength, while 89% is explained by other factors. Since in this study we were only able to investigate a number of parameters it can occur that a significant association can have a P-value of <0.001, while the R-squared value remains low. Also, apart from the relevance of the association, R-squared is also determined by variability in the measurement. It depends on the character of the dependent variable whether such a significant association has biological and/or clinical implications. When associations are described with dependent variables which are mainly determined by genetic factors, which cannot be modified, such a relation may certainly have biological implications. In addition, when only small changes of the dependent variable are necessary to establish clinical changes, such an association will also be of importance.

A. Physical functional ability and Quality of Life

a) Determinants of functional ability

Many of the predictors of physical functional status appear to be potentially modifiable, and research must be done to refine diagnostic criteria and develop practical methods of measurement of key physiological capacities. This in order to identify proper targets for interventions with disabled elderly or of preventive interventions in 'normal' elderly individuals, with the ultimate goal to enhance the proportion of the older population that ages successfully.

Among the physical characteristics measured in this study several relationships were observed as illustrated in Figure 4A. Since it is a cross-sectional study, the arrows in this scheme are theoretical, but were assumed to reflect the most likely direction of cause and effect. For example, it is hypothesized that good muscle strength is necessary to maintain a high physical performance. A person needs a certain amount of leg extensor

strength to be able to rise from a chair. In our study, muscle strength was strongly related to all parts of the physical performance test, but most strongly to the chair stand part. Although the opposite direction is also possible: a good physical performance might lead to a high activity level, which in turn might improve muscle strength. Thus, regarding the results of this study it is hypothesized that muscle strength is a positive determinant of functional ability (measured subjectively by the number of problems in activities of daily living and objectively by physical performance), while fat mass is a negative determinant.

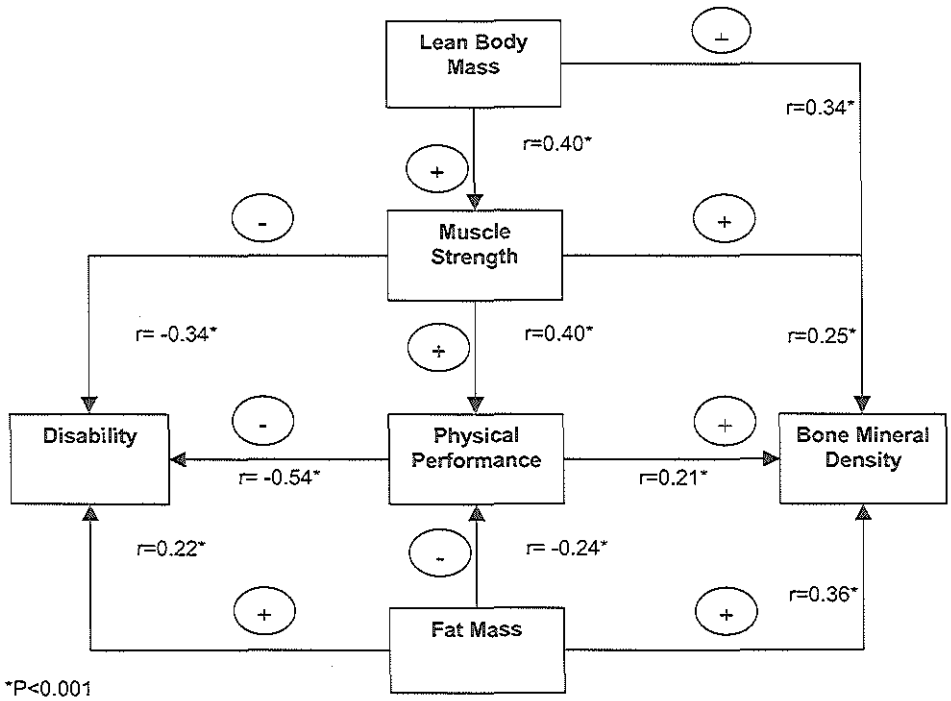


Figure 4A. Correlation between the physical characteristics. r denotes correlation coefficient.

b) How to define chronological and successful aging?

1. Chronological aging

As mentioned earlier, in order to define proper targets for intervention and to establish a prognosis, it is necessary to define what is the physical capacity of an individual in relation to his or her chronological age. So far this is not known. As there are probably large inter-individual differences, while these capacities are probably

dependent on multiple individual and environmental conditions, it is hypothesized that this capacity at least should be that of an older individual free of disease. This capacity then, can serve as a reference for subjects with disease. Since we investigated a relatively healthy population of elderly men, we suggest that we can indeed address the following question in our population. What should we measure to be informed about the "chronological" physical capacity of an older subject? In other words, what is or are the key physical characteristics of aging?

One could measure physical performance according to Guralnik et al. which is an objective measure of functional ability (21). This test was in our population related to nearly all other physical parameters of aging and is probably influenced by them. One could also measure leg extensor strength. It is a parameter, which seems to influence functional ability, but is not a measure of functional ability itself.

The most attractive way, however, to define which is the key physical characteristic of aging in this study seems its the predictive value of death. Impaired activities of daily living (ADL), a low physical performance, muscle strength and bone mineral density were all associated with an increased risk of mortality. The fact that muscle strength and the number of problems in activities of daily living were no longer significantly associated with death after adjustment for physical performance might imply that they were a confounder. On the other hand it might also imply that muscle strength increases physical performance, which is associated with survival, thereby representing only a mechanical pathway. We therefore conclude that muscle strength and functional ability (objectively measured with the physical performance test) are considered the key characteristics of the physical functional status of independently living elderly men. The studies described in this thesis show that measuring muscle strength and physical performance only is probably sufficient in expressing the functional ability in elderly men. Whether this is really sufficient should be verified in longitudinal and intervention studies.

Also, bone mineral density was a very strong predictor of mortality. It is remarkable that even in men who are relatively healthy, this is such a strong predictor. Although we do not have information about the cause of death, this finding might have important consequences for intervention programs. Improving bone mineral density by hormone substitution or strength-enhancing programs, might lead to a decrease in fractures and as a consequence a longer time of independent living until death. Indeed muscle strength and bone mineral density are parameters which might be influenced by intervention therapy directly, like hormonal replacement, while activities in daily living and physical performance can be influenced indirectly by improvement of for example muscle strength, balance and co-morbidity. It still needs to be elucidated whether bone density gives information additive to that of the physical capacity expressed against chronological age.

2. Successful aging

We mentioned earlier in this chapter that we can not fully define and assess successful aging, and it is certainly not possible to assess it in one single measurement. Quality of life (QoL), however, is often used in the context of terms like successful aging

and it might be used as a relatively good indicator. We therefore decided to use it as an end-point in this study. The questionnaire of Henrich and Herschbach (QLS) that we used, includes a large number of aspects (dimensions) of quality of life.

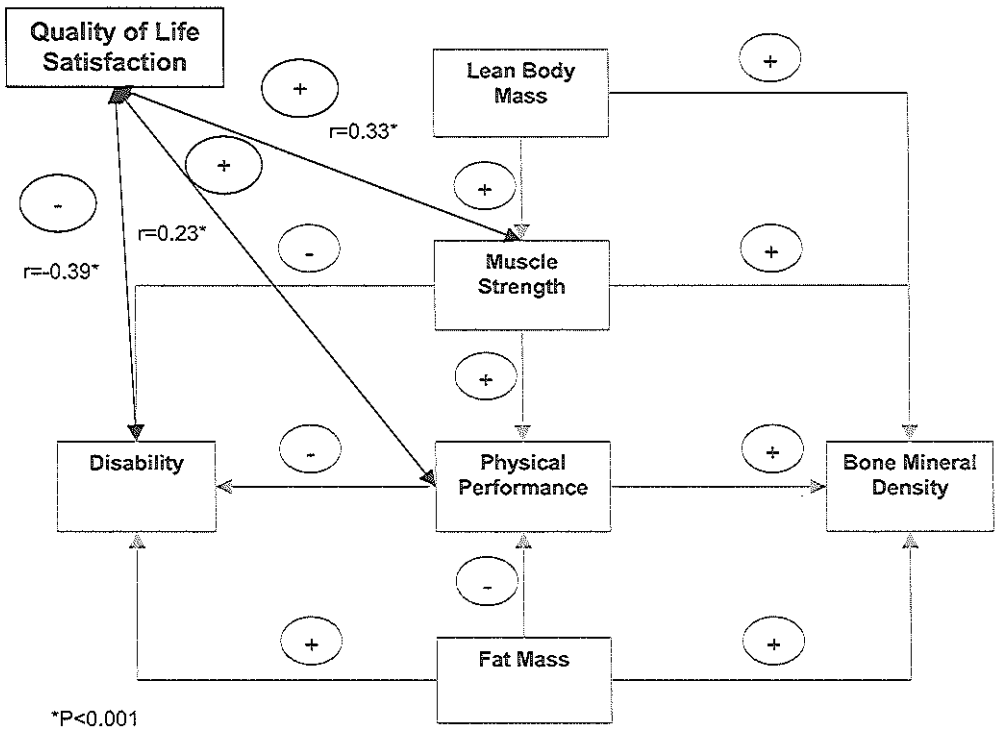


Figure 4B. Correlation between parameters of physical functional ability and quality of life satisfaction. r denotes correlation coefficient.

As expected, physical functional ability was strongly related with QLS (Figure 4B). A large number of problems in activities of daily living as assessed by the Modified Health Assessment Questionnaire was strongly associated with low values of all three parts of QLS. This was not very surprising. Neither very surprisingly was the positive relation between muscle strength and all aspects of the QLS. However, although expected, this has never been demonstrated so clearly before and it emphasizes the importance of maintaining muscle strength in old age. Whether a good quality of life is a consequence of an overall good physical functional status or whether a good quality of life leads to a good physical functional status, cannot be established from the results of this study.

Therefore, an intervention study with for example a training program and with quality of life as outcome measure could be performed. The hypothesis that muscle strength improves quality of life has important implications for intervention strategies, like training programs or hormonal intervention intended to increase muscle strength.

The physical performance score (PPS) was only related to QLS-Health. This suggests that QLS-Health is a parameter which assesses the satisfaction of physical function in a proper manner. The QLS-Health indeed includes questions referring to mobility. However, it remains unclear why PPS is only related to QLS-Health, while muscle strength is strongly related to all three parts. It might be due to the fact that PPS had a skewed distribution and therefore mainly identified individuals with a lower PPS, while muscle strength was normally distributed and therefore was describing subjects in the complete range of physical functional ability. This complete range might be necessary to show the relationship with QoL.

The predictive data on mortality as described above also give additive information on successful aging. In conclusion, the measurement of a combination of both physical performance and muscle strength in older men seems to give the most optimal information about ones' chronological age and his life satisfaction as well. To emphasize the importance of the relationships between the physical characteristics and quality of life, it was demonstrated that subjects with the lowest quality of life had a higher risk of mortality compared to subjects with higher quality of life (Chapter 7). It is important to know, however, whether these relationships might at least in part be explained by lower hormone concentrations as well. After adjustment for both physical functional status and hormone levels, quality of life remained independently related to mortality, however.

B. Endocrine aspects of aging

We demonstrated in this thesis that several significant relationships exist between serum hormone concentrations of the different axes and physiological determinants of functional capacity, such as physical performance, muscle strength, body composition, as well as quality of life. In addition, some endocrine parameters may be used as indicators of physical functional status, as well as predictors of mortality.

a) Somatotropic axis

1. Relation with physical capacity

Remarkably, but in agreement with other studies, serum IGF-I (total nor the free fraction) was not related to measures of physical functional status (56-61). IGF-I might not be a good marker of the activity of the somatotropic axis during aging. In agreement with this, normal serum IGF-I levels are frequently found in elderly individuals with growth hormone (GH)-deficiency (62). It is known that GH itself exerts anabolic effects (63). In addition, serum IGF-I concentrations might not reflect the bio-activity of IGF-I in the different target organs or tissues in a proper manner. Serum IGFBP-2 levels might better represent the integrative effect of growth hormone and IGF-I.

In chapter 2, we described that low serum IGFBP-2 concentrations are highly significantly associated with a better overall physical functional status (Figure 4C). This

observation is new and raises many questions. Longitudinal studies should reveal whether a decrease in functional ability is accompanied by an increase in IGFBP-2 concentrations. How this inverse relation might be explainable and how IGFBP-2 concentrations are regulated has been addressed in chapter 2. In short, IGFBP-2 concentrations are probably determined by the integrated effects of GH secretion, IGF-I and –II levels, insulin secretion, as well as the nutritional state. It is currently unknown whether the presence of (specific) disease(s) influences IGFBP-2 levels. IGFBP-2 concentrations might also be related to the immune system, although they were not associated to the acute phase proteins IL-6 and CRP. The relationship between IGFBP-2 levels and the nutritional state should also be investigated in more detail. These questions can be investigated by assessing the relationship between IGFBP-2 and the nutritional intake. Such a relationship has already been demonstrated in anorexia nervosa patients (64).

Intervention studies with growth hormone might give answers to a number of questions raised by our study. However, we already know from the relatively few double-blind placebo-controlled studies which have been performed previously, that the effect of growth hormone replacement therapy on muscle strength and/or physical performance is minimal in the elderly and that the side-effects are major (65-68).

2.Relation with quality of life

In chapter 7, it was demonstrated that serum IGF-I levels were related to the hormone-related module of the QLS. Also, IGFBP-3 concentrations were positively related to QoL (Figure 4C). After adjustment for one another, both lost their significance with QLS, suggesting that there is an interrelationship between IGF-I, IGFBP-3 and QLS. We did not measure growth hormone secretion in this study. GH is secreted in a pulsatile manner, with maximum secretion during sleep. Therefore one measurement at one time-point is insufficient to be informed about the mean or peak growth hormone concentrations. Instead, we measured serum IGFBP-3 concentrations, which are supposed to be a marker of the mean 24 hour growth hormone secretion. From the fact that both IGF-I and IGFBP-3 loose their significance when adjusted for one another, it can be hypothesized that growth hormone has a beneficial effect on QoL via its activating effect on the secretion of IGF-I. On the other hand, also growth hormone or even IGFBP-3 itself, as well as IGF-I may have independent effects on QoL.

It is known that in growth hormone deficient adults QoL is decreased compared to controls (69). However, it has only once been described previously that serum IGFBP-1 concentrations are inversely related to QoL (70). In our population the inverse relationship between serum IGFBP-1 and QoL, was independent of IGF-I and IGFBP-3, indicating that it was not a confounder. Probably IGFBP-1 levels are an inverse marker of insulin secretion. Higher IGFBP-1 concentrations may also have an unfavorable effect on the development and progression of atherosclerosis (71). The presence of atherosclerosis and cardiovascular disease in turn might be associated with a low quality of life. In this study we adjusted the relationship between quality of life and IGFBP-1 for symptoms of angina pectoris, a previous myocardial infarction and congestive heart

failure. The relation remained significant. Finally, it may also be that IGFBP-1 itself has an inhibitory effect in the brain, opposing the effect of growth hormone and/or IGF-I.

Also IGFBP-2 levels were related to QLS. It seemed, however, that IGFBP-2 is an intermediate or confounder in this association, since after adjustment for parameters of physical ability, it remained no longer significantly related to QLS. Again it cannot be excluded that a more complex relation exists between QoL, the somatotropic axis and physical functional ability.

The effect of growth hormone on QoL has hardly been examined in the elderly population. Therefore intervention programs with growth hormone to increase QoL might be an option to investigate the effect of growth hormone on QoL. However, again it has to be taken into account that it is likely to induce many side-effects as well.

3. Relation with mortality

In chapter 9 it was hypothesized that the predictive value of IGFBP-1 on mortality might be due to the presence of atherosclerosis or insulin resistance. This hypothesis could only be partially examined in this study. Therefore further research is necessary to investigate the role of the somatotropic axis, and specifically IGFBP-1, on mortality.

b) Pituitary-Gonadal axis

1. Relation with physical capacity

With regard to the pituitary-gonadal axis, we demonstrated in chapter 4, that luteinizing hormone (LH), independent of testosterone reflects serum androgen activity in a different way than testosterone, possibly reflecting more closely the combined feedback effect of estrogen and androgen. Testosterone was positively related to muscle strength and bone mineral density and inversely to fat mass. Remarkably no significant association was present with physical performance measured objectively nor with lean body mass. An inverse relation between testosterone and the number of problems in activities of daily living (ADL) became non-significant after adjustment for muscle strength, suggesting that ADL was a confounder or that testosterone is an important determinant of functional ability.

It might therefore be that the measurement of both testosterone and LH will define elderly men who might benefit from testosterone replacement therapy better. It remains to be established whether LH, perhaps in combination with testosterone, might serve as a parameter of the optimal dose and the effect of androgen replacement therapy.

In chapter 3, we demonstrated that non-SHBG-bound testosterone was more strongly associated with muscle strength and bone mineral density than total testosterone. To define whether subjects are hypogonadal it might therefore be better to measure non-SHBG-bound testosterone than total testosterone. This especially applies to elderly men, since serum SHBG concentrations increase during aging. Also very important was our observation that the testosterone / SHBG ratio does not seem to be a good measure in representing the non-SHBG-bound fraction, especially not in a cross-sectional study, since part of the associations found are explained by the inverse association of SHBG. Serum non-SHBG-bound or bioavailable testosterone concentrations were calculated according to the method described by Södergård et al., which is explained in chapter 3

(72). In a computer program this calculation is easy to perform. However, in clinical practice it is suggested that this calculation should be performed in elderly men with clinical symptoms of testosterone deficiency in whom serum total testosterone concentrations do not help defining testosterone deficiency.

After calculating the SHBG-bound, the albumin-bound and the free fraction of testosterone using the "Södergård" method, it appeared that only 35% of the total testosterone was bound to SHBG, while >60% was bound to albumin. According to endocrine text books and recent papers by Vermeulen et al. (50), the percentage testosterone bound to SHBG should be much higher, while the albumin fraction should be much lower. Most of the studies which have investigated the percentages of these fractions have been done in smaller and much younger populations. Still, it is a considerable difference compared to other studies, which we cannot fully explain. The assumption that the calculation is indeed performed in the right way and actually gives the correct percentages is demonstrated by the fact that the SHBG-bound fraction of testosterone was not related to for example muscle strength. However, the only way to be certain that these percentages are correct is to measure bioavailable testosterone in vitro.

It seems that also the non-SHBG-bound fraction of estradiol is superior to total estradiol in describing the relations between estradiol bio-activity and it target organs. These differences between the non-SHBG-bound fraction and total estradiol are, however, less pronounced compared to testosterone. Therefore in future research it should be determined whether it is indeed preferable to measure non-SHBG-bound estradiol.

2. Relation with quality of life

Although it has been demonstrated that in elderly men bioavailable testosterone levels are lower in men with defined depression compared to other men (73), the benefits of testosterone replacement therapy on mood and quality of life have not been well investigated. In our cross-sectional study we demonstrated a positive relationship between bioavailable testosterone and QLS-Health. In addition, we found significant relationships between serum testosterone concentrations and specific questions of the QLS questionnaire, in particular the questions concerning physical condition, mobility, and "independence from help" of the Health-module. This suggests that a potential beneficial effect of testosterone on QoL is mediated via a better physical functional ability. Indeed, the relation between QLS-Health and bioavailable testosterone became non-significant after adjustment for leg extensor strength. However, as mentioned previously, the beneficial effect of testosterone replacement on quality of life should be further investigated in intervention studies.

A remarkable association was found in this study between serum estradiol and QoL. It was thought so far that a potential improvement in well being in women or men was due to their own specific sex steroid. In post-menopausal women, estrogen replacement therapy is known to improve subjective well being (74), and testosterone replacement in hypogonadal men has been reported to improve well being (75). However, in view of the association found in this study, this hypothesis might have to be changed. Although this

association between serum estradiol and QoL in elderly men was found in a cross-sectional study, it might be hypothesized that it is estradiol itself, which exerts the beneficial effect on well-being, directly in the brain or indirectly by improving certain physical characteristics. Future research should focus on the conversion of testosterone to estradiol, and its serum concentrations, during testosterone replacement studies with QoL as outcome measure. In addition, the precise role of estradiol in elderly men must be elucidated in future research; it has to be investigated whether estradiol exerts beneficial effects on other physical characteristics of aging apart from bone mineral density; and whether a potential effect on QoL is a direct effect in the brain.

3. Relation with mortality

Although serum LH, testosterone, and estradiol levels were not directly associated with an increased or decreased risk of mortality four years after the initial investigation, taking the results of the literature as well as the results of this study into account it seems beneficial to replace testosterone in some elderly men. In most studies done so far on testosterone replacement therapy, the beneficial effects seem to be greater than the potential risks. However, only a few studies have investigated properly the effects of testosterone replacement therapy on quality of life and the mechanism of such a potential effect. In addition, it is still unclear which elderly men should be treated with testosterone therapy. Are these men defined by lowered serum testosterone levels or subjects with clinical symptoms of androgen deficiency? How to interpret serum levels and symptoms in the presence of other diseases? Androgen deficiency is very difficult to define, since many symptoms overlap with those of disease and of aging. In conclusion testosterone replacement should be administered only to very well defined groups of otherwise 'healthy' elderly men with clear clinical symptoms of androgen deficiency, in the presence of lowered serum testosterone and/or high LH levels. Also the use of androgen and estrogen receptor modulators might be used to further elucidate the role of estrogens in men.

c) Pituitary-Adrenal axis

1. Relation with physical capacity

In agreement with previous findings, serum dehydroepiandrosterone (DHEA) an its sulfate (DHEAS) both decreased with age in our population. DHEA is sold in the United States in high amounts and has been called the "fountain of youth". Most knowledge concerning the potential function of DHEA, however, was derived from animal studies, mainly from rodents, which do not produce DHEA themselves. Looking at the results of these studies, DHEA (S) is supposed to have a variety of functions, like preventing obesity, cancer, and cardiovascular disease and it might improve physical as well as psychological well being. However, several studies on DHEA replacement in humans yielded inconsistent and controversial results (76-81).

In our population, we have found two significant correlations between DHEAS and the physical characteristics of aging: e.g., a positive relation between DHEAS and physical performance and one with total body bone mineral density (not femoral neck

bone mineral density). Since these relations lost their significance after adjustment for testosterone or estradiol, it is suggested that DHEAS exerts its potential effect through conversion into testosterone and estradiol.

2. Relation with quality of life

Serum DHEAS was positively related to the Health-module of the QLS questionnaire. However, as for the relationship with bone density, this relationship became non-significant after adjustment for estradiol. Whether, DHEA(S) still might have an effect on QoL, through conversion into estradiol, cannot be established from this study.

Serum cortisol concentrations were inversely related to the general module and the hormonal module of QLS. The negative influence of cortisol on the QoL might represent a cumulative deleterious effect of longterm exposure of the human body to relatively high cortisol concentrations, as suggested by McEwen et al. (82). It has be taken into account that only one single measurement of cortisol was done in this study, however.

3. Relation with mortality

Serum DHEAS levels were not linearly related to mortality. However, subjects with DHEAS concentrations in the lowest quartile had a significant higher age-adjusted risk of mortality compared to subjects with the highest DHEAS levels. This might imply that a threshold exists; only very low DHEAS seem to be disadvantageous. This has for example been demonstrated by the fact that women with adrenal insufficiency, and therefore very low DHEA(S) levels, benefit from DHEA therapy (78).

In conclusion, our results combined with data recently published on the potential beneficial effects of DHEA administration in men, which induced a slight improvement on mood scores (79), suggest that this steroid itself only plays a minor role in the aging process. It remains, however, intriguing that DHEAS circulates in the human serum in such high concentrations, probably being a reservoir which is at the tissue level transformed to biologically active sex steroids. The role of DHEA as "fountain of youth" remains far away, however.

d) Pituitary-Thyroidal axis

1.Relation with physical capacity

In chapter 5, we demonstrated that in a subgroup of elderly men low serum reverse triiodothyronine (rT3) and free thyroxine (free T4) concentrations were associated with an overall better physical functional status (Figure 4C). Of course it is known that rT3 levels tend to be elevated in subjects with certain diseases, summarized as 'non-thyroidal illness'. Therefore one may ask whether in our population we are also only describing 'non-thyroidal illness'. However, only 45 subjects met the biochemical criteria for 'non-thyroidal illness'. Remarkably, rT3 levels remained associated with several parameters of physical function in the absence of systemic disease and the low T3 syndrome. Serum rT3 levels may therefore be a marker of physical function in the elderly, whether or not determined by disease or a poor nutritional status.

In the elderly population no studies have been carried out so far on thyroxine sulfate (T4S). Although we demonstrated strong associations of T4S with physical functional characteristics, it is necessary to clarify the importance of T4S measurements both in

older and younger populations. Our study indicates, however, that T4S indeed appears to be an important determinant of the activity of the thyroidal axis. The actual measurement of T4S should be refined, since inter- and intra-assay variability of the assay are considerable.

2.Relation with quality of life

Of the thyroidal axis, serum free T4 and reverse T3 levels were inversely associated with the health section of QLS. The negative relation between serum TSH concentrations and QLS-Health showed a trend towards significance. After adjustment for free T4 or reverse T3, the inverse relation between TSH and QLS-Health became strongly significant. The relationships described between serum rT3, free T4 and TSH and health-related QoL were all independent of the presence of diseases, the use of medications and the physical characteristics of aging. So it appears that a lower activity of the thyroidal axis is associated with a lower health related quality of life. From this cross-sectional study, no conclusions can be drawn with regard to the mechanisms behind these relationships. However, it cannot be excluded that mild thyroid dysfunction has direct negative effects on mood and behaviour, since the previous relationships were all independent. This hypothesis is strengthened by the fact that hypothyroidism is often associated with depression and that subclinical hypothyroidism may be associated with mood disturbances (83). It has to be mentioned, however, that in our population no overt hypothyroidism was present and only 6 subjects had subclinical hypothyroidism (TSH between 4 and 10 IU/l, with normal free F4 levels).

3.Relation with mortality

After adjustment for serum albumin levels, subjects with T3 levels in the lowest quartile of this population had a significantly lower risk of 4-year mortality compared to subjects in the other quartiles. Low free T4 concentrations were also associated with a lower risk of mortality. These remarkable findings are not completely understood at present.

Further, it was expected that reverse T3 would be associated with mortality, since high reverse T3 seemed to be an indicator of disease and a poor physical functional status.

Since these results have not been reported before, they should by confirmed in other studies. In addition, we should focus on the mechanism of the predictive value of serum T3 and free T4 concentrations.

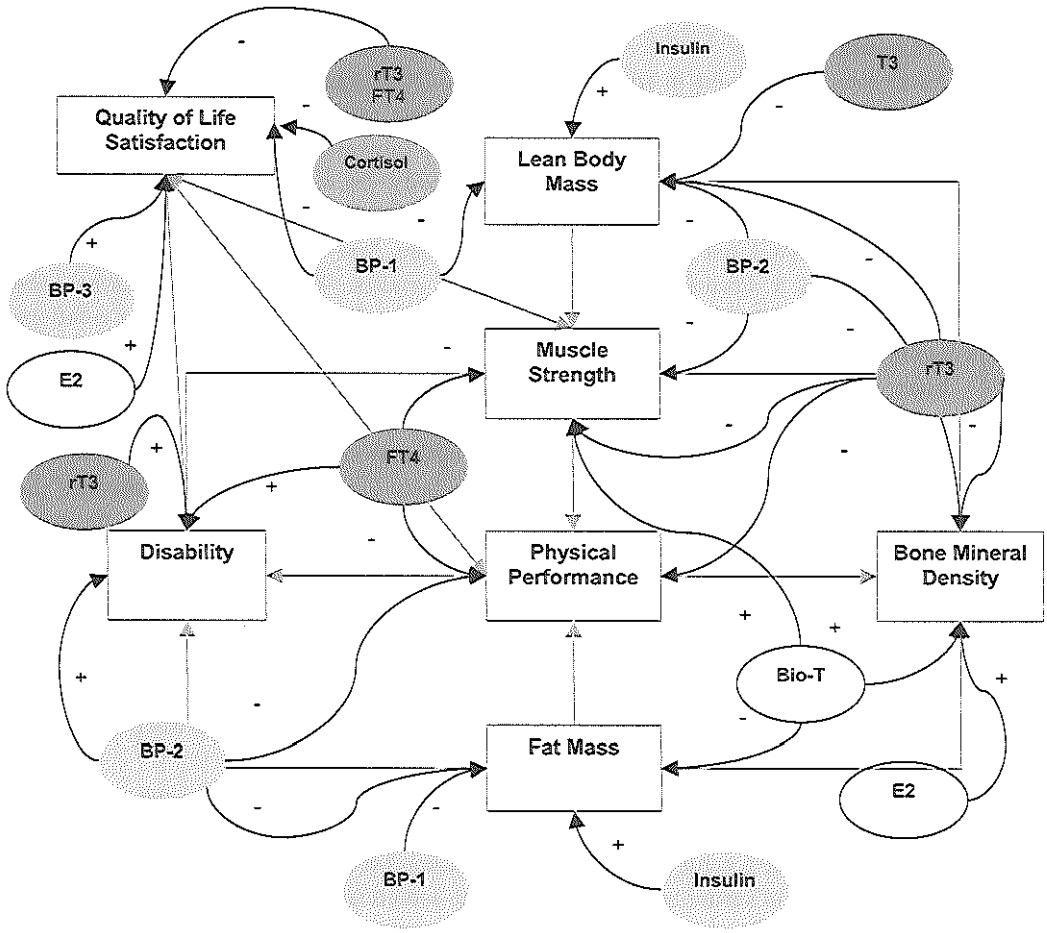


Figure 4C. Associations between hormones and binding proteins of the somatotropic axis, gonadal axis, adrenal axis and thyroidal axis and parameters of physical functional ability and quality of life satisfaction. BP-1 (IGFBP-1), BP-2 (IGFBP-2), BP-3 (IGFBP-3), Bio-T (bioavailable testosterone), E2 (estradiol), T3, (triiodothyronine), FT4 (Free Thyroxine), rT3 (reverse triiodothyronine).

C. Co-morbidity and atherosclerosis

1. Co-morbidity

As already pointed out in the methods section of this chapter, several subjects used medications for certain diseases. The majority was treated with one or more drugs (n=237). It might be hypothesized that all the previously described relations are (partially) dependent on the presence of co-morbidity. Or stated otherwise, high serum IGFBP-2, LH, and reverse T3 levels, and low IGF-I, IGFBP-3, testosterone, estradiol and DHEAS levels would just represent a state of disease. And this state of disease might be associated with a lowered physical functional status. To figure out whether the previously described relationships were independent of co-morbidity, all the relations were adjusted for the parameters of these conditions.

First of all, subjects with for example signs or symptoms of angina pectoris, congestive heart failure, previous myocardial infarction or a cerebrovascular accident, or knee pain indeed had slightly lower muscle strength, lower physical performance, as well as a larger number of problems in activities of daily living. This did not influence the relations between the physical characteristics as illustrated in Figure 4A, however. Subjects with the above mentioned diseases did not have different serum hormone concentrations of the somatotropic, gonadal and adrenal axis. The relationship between the hormones of the thyroidal axis and co-morbidity was discussed in the previous paragraph.

Co-morbidity was also associated with a decreased quality of life as illustrated in chapter 7. Again this association did not influence the relationship between quality of life and physical functional ability or serum hormone levels.

In conclusion, the presence of co-morbidity was associated with a lower physical functional status and a decreased satisfaction of quality of life. However, co-morbidity did not appear to be a confounder in the associations between physical functional status or quality of life and serum hormone levels of the somatotropic, gonadal or adrenal axis.

2. Atherosclerosis

In the context of this thesis we determined the relationship between serum hormone levels and parameters of atherosclerosis. In chapter 6 we describe that serum free IGF-I concentrations as well as testosterone and estrone concentrations are inversely related to the intima-media thickness of the carotid artery. In chapter 6 we also mentioned that in a previous study high fasting serum free IGF-I levels have been associated with a reduced number of atherosclerotic plaques, symptomatic cardiovascular disease and lower serum triglycerides levels (71). In the present study, free IGF-I, rather than total IGF-I, was independently related to the mean intima-media thickness of the carotid bifurcation, suggesting that it is the easily dissociable IGF-I fraction which may be able to act directly on the vascular wall. On the other hand, a potential effect of free IGF-I on the vascular wall may also be indirect via changes in cholesterol levels or insulin sensitivity. However, in this cross-sectional study the relation between free IGF-I and intima-media thickness was not dependent on cholesterol, glucose or insulin levels.

It has never been described previously that testosterone concentrations are related to the intima-media thickness of the carotid artery. First of all, the question is whether testosterone directly influences the vascular wall or indirect via for example changes in the lipid profile or the physical functional ability. The relation between testosterone and intima-media thickness was independent of serum cholesterol concentrations. However, a definitive answer to this question may only be given by performing a placebo-controlled trial with testosterone administration, and monitoring the effect on intima-media thickness. Secondly, it can be asked whether, as with IGF-I, serum free or non-SHBG-bound testosterone levels are more strongly related to the intima-media thickness. This was not the case in our study (data not shown).

3. Parameters of inflammation

It has been demonstrated before that C-reactive protein and interleukin-6 (IL-6) concentrations reflect inflammation and predict cardiovascular disease as well as mortality in a healthy, non-disabled populations with or without previous cardiovascular disease (6,7). In our population serum CRP levels only predicted mortality in subjects with prevalent cardiovascular disease. IL-6 levels, on the other hand, predicted mortality in those with and without cardiovascular disease. IL-6 levels, within the normal range, were the strongest biochemical predictor of mortality in our population of independently living older men, independent of disease, bone density and disability, cortisol, insulin, CRP and albumin levels and smoking. It cannot be established from the results of this study, whether low IL-6 serves merely as an indicator of subjects with a lower risk of mortality or whether it plays a role in a physiological pathway.

It has also been described that the combination of low IGF-I and high IL-6 levels predicted disability and death in a population of older women (84) and that high IL-6 levels predict a greater cognitive decline in elderly men and women (85). Therefore it should be investigated whether IL-6 not only predicts mortality but also a lower physical functional capacity and cognition. In addition, it could be investigated whether other biomarkers act synergistically with IL-6 to predict mortality.

5. CONCLUSIONS

In an attempt to gain a greater understanding of the process of aging and its determinants, the concepts "frailty" and "successful aging" have become popular. Frailty focuses mainly on the physical aspects of aging, while the concept of successful aging includes a much broader range of aspects of life, such as physical, psychological and social aspects. In this thesis the definition of successful aging as illustrated in the model by Rowe (Figure 1) was taken as a starting point in order to gain insight in the role and the interactions of the different physical and endocrine factors which are assumed to be important to age successfully. Rowe developed a definition of successful aging with 'maintaining high cognitive and physical functional capacity', 'avoiding disease and disability', and 'maintaining active engagement with life' as main determinants.

However, for an individual assessment of successful aging adequate, reproducible, and if possible predictive measures are needed. Consequently one of the aims of our study was to find parameters which reliably reflect the three different parts of the model. To measure overall successful aging we used two parameters; quality of life and survival.

Maintaining an optimal cognitive and physical functional capacity is one of the three main parts of the model. In our population cognition was relatively good, since most of the men had a high score on the 'Mini-Mental State Examination'. With regard to physical functional capacity it is suggested that measuring physical performance according to the method described by Guralnik et al. and muscle strength might be sufficient to be informed about this aspect. However, physical performance may not be modified directly by interventions, but is more likely modified by changes in muscle strength, balance, bone mineral density and perhaps body composition. In order to select those who might benefit from intervention therapy and to monitor therapy, it may be useful to measure muscle strength and bone mineral density rather than, or in combination with physical performance.

Avoiding disease and disability is the second component of the model by Rowe. In this population, men with certain diseases had a lower functional ability, measured subjectively as well as objectively. This indicates the correctness of the model. It is suggested that (subclinical) atherosclerosis is also an aspect of this part of the model. Reducing the atherosclerotic process might lead to a decrease in cerebro- and cardiovascular events, which in turn helps to age successfully.

Finally, maintaining active engagement with life is the third part of the model by Rowe. It was tried to cover this part at least in part by measuring loneliness. Physical performance and loneliness were inversely related indicating the overlap between the active engagement in life part and the physical functional capacity part. In addition, subjects that were lonely were significantly less satisfied with life.

Most aspects measured in the different parts were related to quality of life and survival, indicating the combined area of successful aging (Figure 6).

An important aim of this study was to investigate the role of the endocrine system and parameters of inflammation on the different parts of successful aging. Low serum IGFBP-2, luteinizing hormone and reverse T3 and high serum testosterone and estradiol concentrations were related to several aspects of a better physical functional capacity. High testosterone, estrone and free IGF-I levels were related to a lower degree of atherosclerosis. Further, high serum IGFBP-3 and estradiol levels and low IGFBP-1, cortisol and reverse T3 levels were associated with a better life satisfaction. Finally, low IGFBP-1, cortisol, T3 and free T4 concentrations were associated with a lower risk of mortality. This indicates that each endocrine axis has one or more parameters which are related to successful aging (Figure 7). However, whether these parameters directly influence the aging process or whether these are markers of a decreased activity of the axis and in turn potentially influence the aging process can not be fully established form the results of this study. Follow-up and intervention research is necessary to address these questions.

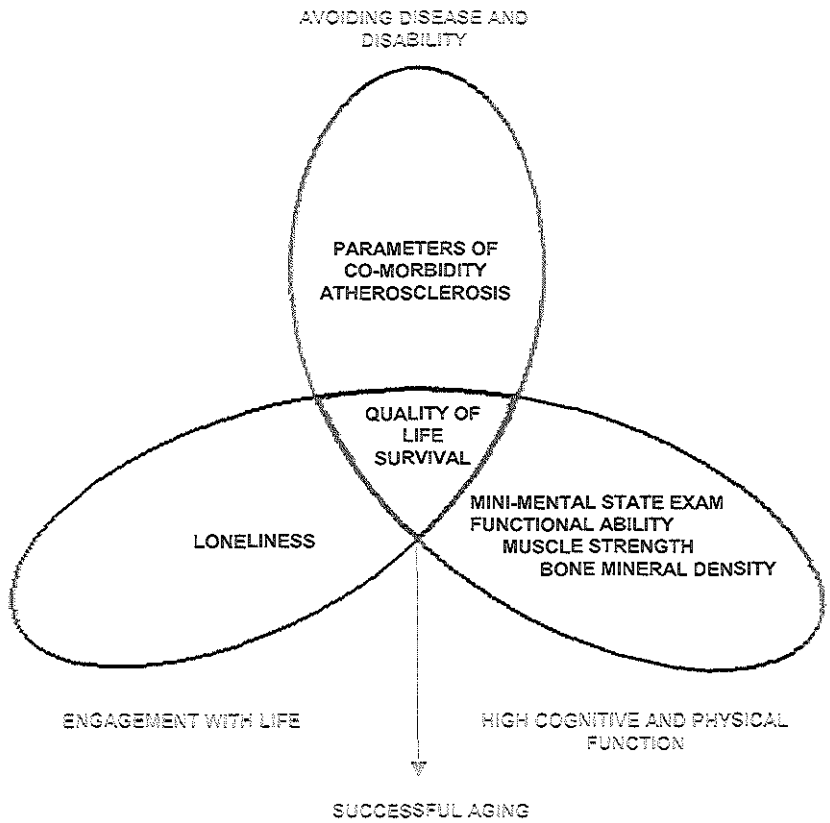
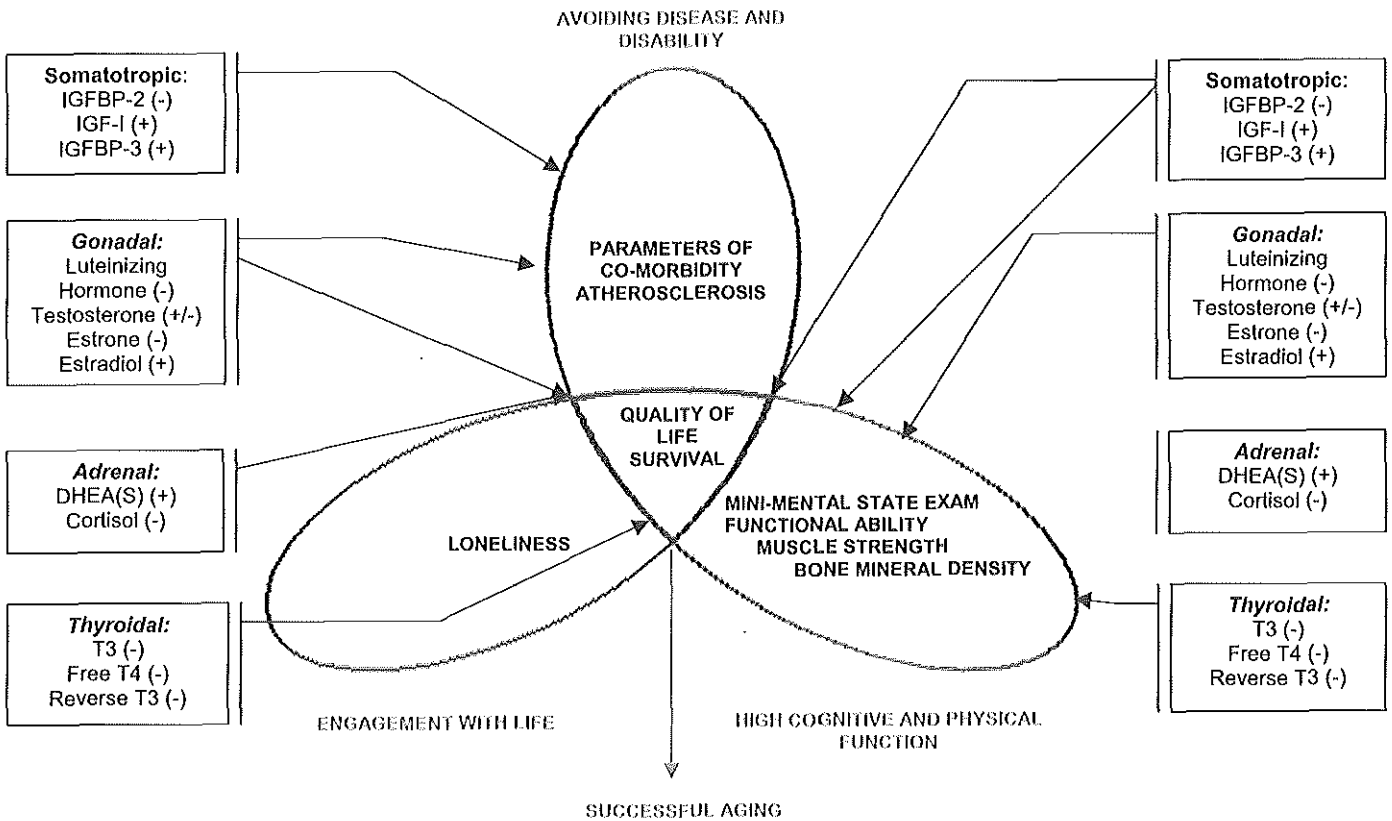


Figure 5. Parameters measured in our study of 403 elderly men implemented in the model of 'Successful Aging' by Rowe.

High serum C-Reactive Protein strongly predicted 4-year mortality, while low interleukin-6 levels predicted 4-year survival. So far we have not investigated potential relations with the different parts of the model of successful aging. Although the predictive value of CRP and IL-6 on mortality was independent of subclinical atherosclerosis and physical capacity, the physiological pathway towards the predictive value on mortality should be further elucidated.

Figure 6. Parameters of each endocrine axis in relation to 'Successful Aging'.



A number of other questions remain to be addressed. Does the decrease in activity of these endocrine axes have one major set point? In other words, does a central pacemaker of endocrine aging exist? And secondly, do the hormones of the different axes influence one another? The results of our study suggest that all these previously described parameters of the different axes are independent of one another in their relationship to physical function, co-morbidity (and atherosclerosis), quality of life and survival and thus successful aging. These are important observations, since this implies that subjects, who might benefit from hormonal intervention therapy, can be selected according to the axis which is 'insufficient' during aging. In addition, combination therapies of two different hormones, might be an option. In a longitudinal study the strength of the decline or increase of hormonal parameters of the different axes studied should be compared to observe whether the decrease of the activity of the different axes occur in parallel.

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CHAPTER 11

/ Samenvatting. *Summary / Samenvatting.* Summary / Samen

SUMMARY

Overall, the aim of this thesis was to obtain more insight in the relations between the endocrine system and the physical and psychological aging process in elderly men. In **chapter 1** an overview is given about the current knowledge on the endocrine system and the aging process. The selection of the participants to this study included those that lived independently, maintaining a reasonable degree of physical capacity and a relatively good cognition. This allowed us to approach the concept of 'successful aging'. In order to do so, a number of questions were addressed in a population of 403 men aged between 73 and 94 years:

1) Since it is difficult to define those subjects that age successfully with respect to physical function, in **chapter 2** we tried to determine which are the main physiological determinants of functional ability in the elderly. Muscle strength and functional ability (measured subjectively as the number of problems in activities of daily living and objectively as physical performance) are likely to be the key characteristics of physical functional status in independently living elderly men, since they were so strongly related to the other physical characteristics measured in this study. In addition, in **chapter 9** it is demonstrated that functional ability, muscle strength and bone mineral density were strongly related to 4-year mortality. Muscle strength and bone mineral density may be markers for general health or a low muscle strength and bone density may lead to imbalance and an increase in fractures and as a consequence mortality. Further, in **chapter 7** the importance of muscle strength and functional ability is emphasized by the fact that they were strongly positively related to quality of life, which was measured using a validated questionnaire consisting of three modules of 8 questions each, directed at general factors (QLS-General), health factors (QLS-Health), and factors related to hormone deficiency (QLS-Hormone).

2) Secondly, since the age-related decline in serum hormone levels occurs in parallel with the loss of physical function, bone mass, muscle mass and muscle strength, it was investigated whether serum hormone levels of four endocrine axes were related to the physical functions measured, including bone mineral density and body composition. In **chapter 2** it was investigated whether components of the somatotropic system contribute to the maintenance of functional ability. Low serum IGFBP-2 concentrations seem to be a powerful indicator for an overall good physical functional status, since IGFBP-2 concentrations were negatively related to activities in daily living, physical performance, muscle strength, bone mineral density of proximal femur, lean mass and fat mass. IGFBP-2 probably inversely reflects the integrated sum of nutritional status as well as of the biological effects of growth hormone, IGF-I, and insulin. Serum insulin concentrations were positively and IGFBP-1 concentrations negatively related to lean mass and fat mass.

In **chapter 3** we tested the hypothesis that the changes in bone mass, body composition and muscle strength with age are related to the fall in circulating endogenous testosterone and estrogen concentrations. Serum testosterone was positively related with muscle strength and bone mineral density. An inverse association existed

between testosterone and fat mass. Independent of testosterone, estrone and estradiol were both positively associated with bone mineral density.

Thyroid hormones regulate the metabolic thermostat by stimulation of the basal metabolic rate. Therefore physiological changes in thyroid hormone concentrations with aging might influence the overall physical functional status. In **chapter 5** we investigated to what extent thyroid hormone concentrations are related to physical functional status. Serum reverse T3 appeared to be positively related to the number of problems in activities of daily living and inversely to physical performance, muscle strength, and bone mineral density. Also, serum free T4 levels were inversely related to physical performance, and grip strength and positively to the number of problems in activities of daily living. Serum T3 and T4 concentrations were not significantly related to physical functional status. T3 was inversely related to lean mass. Higher serum rT3 concentrations result from a decreased peripheral metabolism of thyroid hormone and have been demonstrated to reflect a catabolic state. The relation between rT3 and physical functional status may reflect the presence of disease or a poor nutritional state.

Since part of the age-related physiological changes seem to be influenced by the activity of the endocrine system, one could hypothesize that serum hormone concentrations not only relate to measures of functional ability, but also predict mortality. In **chapter 9**, it was defined which parameter of each axis was predictive of survival or mortality. Of the gonadal, adrenal, somatotropic and thyroidal hormones, high serum IGFBP-1, free T4 and T3 concentrations all predicted 4-year mortality. These hormonal parameters appear to be well informative to establish the mortality risk, independent of co-morbidity and atherosclerosis. Interestingly, most concentrations were within the normal range.

In **chapter 7**, it was established whether serum hormone concentrations of the endocrine axes were related to quality of life. Serum bioavailable testosterone weakly correlated to QLS-Health, while DHEA(S) did not. Serum estradiol and IGFBP-3 concentrations were strongly positively associated to all QLS modules, while cortisol and IGFBP-1 were inversely related with QLS-General and QLS-Hormone. In addition, IGFBP-1 was also inversely related to QLS-Health. Serum IGF-I concentrations were weakly positively associated with QLS-Hormone only. These data, although collected in a cross-sectional study indicate that hormonal intervention, which aims at increasing estradiol bio-availability and/or growth hormone levels might further improve quality of life in elderly independently living men. The importance of defining determinants of quality of life is shown by the fact that quality of life itself is an important predictor of mortality.

In **Chapter 8** the main study objective was to examine factors associated with successful aging amongst older men. By means of different instruments and questionnaires, we operationalised selected aspects of Rowe and Kahn's model of successful aging. Despite various associations on bivariate analysis, health-related quality of life measured by the EuroQol EQ-5D instrument (with 5 dimensions: mobility, self care, usual activities, pain/discomfort and anxiety/depression) was associated with only activities of daily living (ADL) and loneliness on multiple regression. ADL and

loneliness in turn were both associated with physical performance level. Serum IGFBP-3, which reflects growth hormone bioactivity, as well as testosterone concentrations were both strongly related to a better quality of life measured by the EuroQol.

3) Because, serum hormones are mainly bound to their hormone binding globulins and albumin, it remained to be established whether the bioavailable hormone fraction is the best representation of the bioactive hormone concentration. Bioavailable or non-SHBG-bound and free testosterone and estradiol were calculated according to a measure described by Södergård. In **chapter 3** it is demonstrated that non-SHBG-bound and free testosterone were more strongly related to muscle strength, bone mineral density and fat mass than total testosterone and were also significantly related to hip bone mineral density, while total testosterone was not. Non-SHBG-bound testosterone seems to be the best parameter for serum levels of bioactive testosterone, which appears to play a direct role in the various physiological changes which occur during aging. Non-SHBG-bound estradiol was slightly stronger related to bone mineral density than total estradiol. Therefore, non-SHBG-bound estradiol seems to be the best parameter of serum bioactive estradiol in describing its positive relation with bone mineral density.

4) Since recently a common variant form of luteinizing hormone (LH) was detected in apparently healthy individuals, caused by point mutation-based substitutions of two amino acids (Trp8Arg and Ile15Thr) in the LHβ subunit and since it was suggested that the in vivo bioactivity of the LH variant is lower than that of the wild-type hormone, it was determined in **chapter 4** whether differences in bioactivity of variant LH were related to characteristics of physical functional ability. 12.5% of the study population was heterozygous for the LH variant allele. LH concentrations were inversely related to muscle strength and lean mass and both relations were independent of testosterone. LH was positively related to self-reported disability. LH seems to reflect serum androgen activity in a different way than testosterone, possibly reflecting more closely the combined feedback effect of estrogen and androgen. Testosterone levels and the degree of frailty were not different in the wild-type LH group compared with the heterozygote LH variant group. However, as a relatively higher proportion of subjects heterozygous for the LH variant allele had high LH and low T levels, the two LH forms may differ in biological response, in favor of wild-type LH.

5) Since atherosclerosis is an important cause of cardiovascular morbidity and mortality during aging, and as changes in endocrine systems may influence the atherosclerotic process, the relationships between plasma hormone concentrations and parameters of atherosclerosis were determined in **chapter 6**. Serum testosterone, estrone and free IGF-1 were inversely related to intima-media thickness of the carotid artery, also after adjustment for age and body mass index. Free IGF-I, rather than total IGF-I, was independently related to the mean intima-media thickness of the carotid bifurcation, suggesting that it is the easily dissociable IGF-I fraction which may be able to act on the vascular wall. Serum estradiol, DHEA(S), IGFBP-1, -2, -3 and leptin showed no association with parameters of atherosclerosis.

6) It has been demonstrated before that C-reactive protein (CRP) and interleukin-6 (IL-6) concentrations reflect inflammation and predict cardiovascular disease as well as

mortality in a healthy, non-disabled population with or without cardiovascular disease. Also in our population high CRP and IL-6 were strongly related to mortality as decribed in **chapter 9**. Low IL-6 and CRP levels in the normal range may therefore be indicators for the absence of (subclinical) disease in general in the very old.

To be informed about one's individual condition and to select those who may benefit from intervention, the use and knowledge of modifiable parameters that predict survival or mortality may be useful. In **chapter 9**, apart from the well-known conditions like smoking, co-morbidity and intima-media thickness of the carotid artery, the relative importance of a range of potential physical, endocrine and inflammatory predictors of mortality were addressed. Low physical performance, muscle strength and bone mineral density, high serum IGFBP-1, free T4 and T3 concentrations all predicted mortality. Finally, low serum albumin and high C-reactive protein and interleukin-6 levels were associated with a higher mortality rate. With the seven most significant parameters 78% of subjects could be correctly identified with respect to alive or death after four years .

7) Finally, in the discussion described in **chapter 10** an attempt was made to integrate the results of this thesis into the concept of successful aging. The definition of successful aging as defined by Rowe was taken as a starting point in order to obtain more insight in the value and the interactions of the different aspects which are important to age successfully. Rowe developed a concept of successful aging with 'maintaining high cognitive and physical functional capacity', 'avoiding disease and disability', and 'maintaining active engagement with life' as main determinants. However, for an individual assessment of successful aging adequate, reproducible, and if possible predictive measures are needed. Therefore one of the aims of our study was to find parameters which reliably reflect the three different parts of the model. To measure overall successful aging we used two parameters: quality of life and survival.

Maintaining a high cognitive and physical functional capacity is one of the three main parts of the model. In this population cognition was relatively good, since most of the men had a high score on the 'Mini-Mental State Examination'. With regard to physical functional capacity it is suggested that measuring physical performance according to the method described by Guralnik, and muscle strength might be sufficient to be informed about this aspect.

Avoiding disease and disability is the second part of the model by Rowe. In this population, men with certain diseases had a lower functional ability, measured subjectively as well as objectively. This indicates the correctness of the model. It is suggested that (subclinical) atherosclerosis is also an aspect of this part of the model.

Finally, maintaining active engagement with life is the third part of the model by Rowe. Physical performance and loneliness were inversely related indicating the overlap between the active engagement in life part and the physical functional capacity part. In addition, subjects who were lonely were significantly less satisfied with life.

Most aspects measured of the different parts were related to quality of life and survival, indicating the combined area of successful aging.

SAMENVATTING

Het algemene doel van dit proefschrift was het verkrijgen van meer inzicht in de relaties tussen het endocriene systeem en het fysieke en psychologische verouderingsproces bij mannen. In **hoofdstuk 1** wordt een overzicht gegeven van de huidige kennis van het endocriene systeem tijdens veroudering. Criteria voor de selectie van proefpersonen voor deze studie waren zelfstandig wonen en het hebben van een redelijk goede fysieke en cognitieve capaciteit. Dit maakte het bij benadering mogelijk het concept 'succesvol ouder worden' te onderzoeken. Hiervoor werden een aantal vraagstellingen geformuleerd en onderzocht in een populatie van 403 mannen met een leeftijd tussen de 73 en 94 jaar:

1) Aangezien het moeilijk is die mannen te selecteren die succesvol ouder worden met betrekking tot hun fysieke functie, hebben we in **hoofdstuk 2** getracht aan te tonen wat de belangrijkste determinanten zijn van de fysieke functionele status bij ouderen. Spierkracht en functioneel vermogen (welke subjectief werd gemeten als het aantal problemen in activiteiten in dagelijks leven en objectief als fysieke prestatie) zijn waarschijnlijk de belangrijkste kenmerken van de fysieke functionele status bij zelfstandig wonende oudere mannen. Bovendien wordt in **hoofdstuk 9** aangetoond dat functioneel vermogen, spierkracht en botdichtheid sterk gerelateerd zijn aan de 4-jaars overleving. Spierkracht en botdichtheid zouden markers kunnen zijn voor de algemene conditie. Een lage spierkracht en botdichtheid zouden ook kunnen leiden tot een verhoogde kans op vallen en dientengevolge een verhoogd risico op fracturen en overlijden. Verder wordt in **hoofdstuk 7** het belang van spierkracht en het functioneel vermogen benadrukt aangezien bleek dat beide sterk aan kwaliteit van leven zijn gerelateerd. Kwaliteit van leven werd gemeten middels een gevalideerde vragenlijst bestaande uit drie modules van elk 8 vragen, die gericht zijn op algemene factoren (QLS-General), gezondheidsfactoren (QLS-Health) en factoren die gerelateerd zijn aan hormoon deficiënties (QLS-Hormone).

2) Omdat de leeftijdsgerelateerde daling van serum hormoon concentraties parallel verloopt met het verlies van functioneel vermogen, botdichtheid en spierkracht, werd onderzocht of hormoon concentraties van de verschillende endocriene assen gerelateerd waren aan de gemeten fysieke functies alsmede aan botdichtheid en lichaamssamenstelling. In **hoofdstuk 2** hebben we onderzocht of componenten van het somatotrope systeem bijdragen aan het behoud van het functionele vermogen. Een lage serum IGFBP-2 concentratie lijkt een sterke indicator te zijn voor een algehele goede fysieke functionele status, omdat deze negatief gerelateerd was aan de activiteiten in het dagelijks leven, de fysieke prestatie, spierkracht, botdichtheid van het proximale femur, spiermassa en vetmassa. IGFBP-2 reflecteert waarschijnlijk invers de geïntegreerde som van de voedingsstatus en de biologische effecten van groeihormoon, IGF-I en insuline. Serum insuline concentraties waren positief en IGFBP-1 concentraties negatief gerelateerd aan spiermassa en vetmassa.

In **hoofdstuk 3** werd de hypothese getest dat veranderingen in botmassa, lichaamssamenstelling en spierkracht die optreden tijdens veroudering, gerelateerd zijn

aan de daling in circulerende testosteron en oestrogeen concentraties. Serum testosteron was positief gerelateerd aan spierkracht en botdichtheid. Een inverse associatie was aanwezig tussen testosteron en vetmassa. Onafhankelijk van testosteron, waren oestradiol en oestron beide positief geassocieerd met botdichtheid.

Schildklierhormonen reguleren de metabole thermostaat door stimulatie van het basaal metabolisme. Derhalve zouden fysiologische veranderingen in schildklierhormoon concentraties tijdens veroudering wellicht de fysieke functionele status kunnen beïnvloeden hetgeen werd onderzocht in **hoofdstuk 5**. Serum reverse T3 bleek positief gerelateerd te zijn aan het aantal problemen in activiteiten van het dagelijks leven en invers aan de fysieke prestatie, spierkracht en botdichtheid. Hogere serum reverse T3 concentraties zijn het gevolg van een verminderd perifeer metabolisme van schildklierhormoon en het is aangetoond dat dit zou kunnen wijzen op een verhoogd katabolisme. De relatie tussen reverse T3 en de fysieke functionele status zou deels de aanwezigheid van ziekte of een slechte voedingsstatus kunnen representeren. Ook serum vrij T4 was invers gerelateerd aan de fysieke prestatie en knijpkracht en positief aan het aantal problemen in activiteiten van het dagelijks leven. De totale T3 en T4 concentraties waren niet significant gerelateerd aan de fysieke functionele status. T3 was alleen invers gerelateerd aan spiermassa.

Aangezien de leeftijdsgerelateerde fysiologische veranderingen deels beïnvloed lijken te worden door de activiteit van het endocriene systeem, zou men kunnen veronderstellen dat serum hormoon concentraties niet alleen gerelateerd zijn aan functioneel vermogen, maar dat zij ook mortaliteit voorspellen. In **hoofdstuk 9** werd onderzocht of parameters van de gonaden-, bijnier-, somatotrope- en schildklier-as voorspellend waren voor overleving of overlijden. Hogere serum IGFBP-1, vrij T4 en totaal T3 concentraties voorspelden allen de 4-jaars mortaliteit. Deze hormonale parameters lijken zeer informatief voor het vaststellen van het overlijdensrisico, onafhankelijk van co-morbiditeit en atherosclerose. Opmerkelijk was dat de meeste concentraties binnen de normaal waarden voor gezonde volwassen lagen.

In **hoofdstuk 7** werd onderzocht of serum hormoon concentraties gerelateerd waren aan kwaliteit van leven. De bioactieve testosteron fractie was zwak gecorreleerd met het QLS-Health, terwijl DHEA(S) niet gerelateerd was aan kwaliteit van leven. Serum oestradiol en IGFBP-3 concentraties waren sterk positief gerelateerd aan alle modulen van de kwaliteit van leven vragenlijst, terwijl cortisol en IGFBP-1 invers gerelateerd waren aan QLS-General en QLS-Hormone. Bovendien was IGFBP-1 ook invers gerelateerd aan QLS-Health. Serum IGF-I concentraties waren zwak positief geassocieerd met alleen QLS-Hormone. Deze gegevens, ofschoon verzameld in een cross-sectionele studie, suggereren dat hormonale interventie, gericht op het verhogen van oestradiol beschikbaarheid en/of groeihormoon concentraties, de kwaliteit van leven van zelfstandig wonende oudere mannen zou kunnen verbeteren. Het belang van het aantonen van determinanten van kwaliteit van leven blijkt uit het feit dat kwaliteit van leven zelf een belangrijke voorspeller van mortaliteit is.

Het belangrijkste doel in **hoofdstuk 8** was het onderzoeken van factoren die geassocieerd zijn met succesvol ouder worden bij oudere mannen. Geselecteerde aspecten

van het 'succesvol ouder worden' model van Rowe en Kahn werden onderzocht. Ondanks verschillende associaties bij bivariate analyse, was bij multivariate analyse de gezondheidsgerelateerde kwaliteit van leven (welke gemeten werd middels de EuroQol EQ-5D vragenlijst bestaande uit 5 dimensies: mobiliteit, zelfverzorging, dagelijkse activiteiten, pijn/ongemak en angst/depressie) alleen geassocieerd met het aantal problemen in activiteiten van het dagelijks leven (ADL) en eenzaamheid. Problemen in ADL en eenzaamheid waren beide invers geassocieerd met fysieke prestatie. Ook serum IGFBP-3 concentraties, welke de groeihormoon bioactiviteit reflecteren, en testosteron concentraties waren beide sterk gerelateerd aan een betere kwaliteit van leven gemeten met de EuroQol.

3) Omdat serum hormoon concentraties met name gebonden zijn aan hun specifieke transport eiwitten en albumine, moest nog worden vastgesteld of de vrije hormoon fractie de beste weerspiegeling is van de bioactieve hormoon concentraties. Niet-SHBG-gebonden en vrij testosteron en oestradiol werden berekend volgens een methode welke is beschreven door Södergård. In **hoofdstuk 3** wordt aangetoond dat niet-SHBG-gebonden en vrij testosteron sterker gerelateerd waren aan spierkracht, botdichtheid en vetmassa dan totaal testosteron en dat zij ook gerelateerd waren aan heup botdichtheid, terwijl totaal testosteron dat niet was. Niet-SHBG-gebonden testosteron lijkt de beste parameter voor de bioactieve testosteron concentratie, en lijkt een directe rol te spelen bij verschillende fysiologische veranderingen tijdens veroudering. Niet-SHBG-gebonden oestradiol was iets sterker gerelateerd aan botdichtheid dan totaal oestradiol. Daarom lijkt niet-SHBG-gebonden oestradiol de beste parameter voor de bioactieve oestradiol concentratie in het beschrijven van de relatie met botdichtheid.

4) Recent is een variant van het luteïniserend hormoon (LH) gevonden in ogenschijnlijk gezonde individuen, veroorzaakt door substitutie van twee aminozuren (Trp8Arg and Ile15Thr) ten gevolge van puntmutaties in de LHβ subunit. Aangezien de in vivo bioactiviteit van deze LH variant mogelijk lager is dan die van het wild-type, hebben we in **hoofdstuk 4** bepaald of de verschillende LH varianten gerelateerd waren aan de kenmerken van het fysieke functionele vermogen. 12,5% van de studie populatie was heterozygoot voor het LH variant allel. LH concentraties in de hele populatie waren invers gerelateerd aan spierkracht en spiermassa en beide relaties waren onafhankelijk van testosteron. LH was positief gerelateerd aan het aantal problemen in activiteiten van het dagelijks leven. LH weerspiegelt mogelijk de serum androgeen activiteit op een andere wijze dan testosteron, waarbij de LH concentratie wellicht het gezamenlijke terugkoppelingseffect van androgenen en oestrogenen reflecteert. Testosteron concentraties en de mate van fragiliteit waren niet verschillend tussen de wild-type groep en de heterozygote LH variant groep. Echter, omdat een relatief hoog aantal van de mannen die heterozygoot waren voor het LH variant allel, hoge LH en lage testosteron concentraties hadden, zouden de twee vormen van LH kunnen verschillen in hun biologische respons, in het voordeel van het wild-type LH.

5) Omdat atherosclerose tijdens veroudering een belangrijke oorzaak is van cardiovasculaire morbiditeit en mortaliteit, en omdat veranderingen in endocriene systemen het atherosclerotische proces zouden kunnen beïnvloeden, werden in

hoofdstuk 6 de relaties tussen hormoon concentraties en maten van atherosclerose bepaald. Serum testosteron, oestron, en vrij IGF-I waren invers gerelateerd aan de intima-media dikte van de arteria carotis, ook na correctie voor leeftijd en BMI. Met name het vrije IGF-I i.p.v. het gebonden IGF-I, was onafhankelijk gerelateerd aan de intima-media dikte van de carotis bifurcatie, wat suggereert dat de vrije IGF-I fractie mogelijk anti-atherogene effecten heeft op de bloedvatwand. Serum oestradiol, DHEA(S), IGFBP-1, -2 en -3 en leptine waren niet geassocieerd met parameters van atherosclerose.

6) Het is reeds aangetoond dat C-reactive protein (CRP) and interleukine-6 (IL-6) concentraties een ontstekingsreactie weerspiegelen en dat zij beide voorspellend zijn voor mortaliteit in een gezonde, niet geïnvalideerde populatie met en zonder cardiovasculaire ziekte. Zoals beschreven in **hoofdstuk 9**, waren ook in onze populatie hoge CRP en IL-6 concentraties gerelateerd aan mortaliteit. Lage IL-6 en CRP concentraties binnen de normaal waarden zouden daarom bij ouderen kunnen wijzen op de afwezigheid van (subklinische) ziekte.

Om iemands individuele conditie te beoordelen en om diegenen te selecteren die mogelijk baat kunnen hebben bij interventie therapie, is het wenselijk over parameters te beschikken die mortaliteit of overleving voorspellen. In **hoofdstuk 9** wordt, behoudens de bekende condities zoals roken, ziekte en atherosclerose, het relatieve belang van een scala aan potentiële fysieke, endocriene en inflammatoire voorspellers van mortaliteit besproken. Een lage fysieke prestatie, spierkracht en botdichtheid, hoge serum IGFBP-1, vrij T4 en totaal T3 concentraties waren allen voorspellend voor een verhoogd risico op overlijden. Ook lage albumine en hoge CRP en IL-6 concentraties waren geassocieerd met een verhoogde mortaliteit. Met de zeven meest significante voorspellers kon van 78% van de mannen de mortaliteit na vier jaar correct voorspeld worden.

7) Ten slotte, in de discussie zoals beschreven in **hoofdstuk 10**, werd getracht om alle resultaten van dit proefschrift te integreren in het concept van succesvol ouder worden. De definitie van succesvol ouder worden zoals beschreven door Rowe werd als uitgangspunt genomen om meer inzicht te verkrijgen in de verschillende aspecten die belangrijk zijn voor het succesvol ouder worden. Rowe ontwikkelde een concept van succesvol ouder worden met 'het behoud van een goed cognitief en fysiek functioneel vermogen', 'het vermijden van ziekte en handicap', en 'het behoud van een actieve betrokkenheid met het leven' als belangrijkste determinanten. Echter voor een individuele beoordeling van succesvol ouder worden zijn adequate, reproduceerbare en indien mogelijk voorspellende maten nodig. Daarom was een van de doelen van onze studie om parameters te vinden die betrouwbaar de drie verschillende onderdelen van het model van Rowe reflecteren. Om succesvol ouder worden in zijn algemeenheid te meten hebben we twee parameters genomen: kwaliteit van leven en overleving.

Het behouden van een goede cognitieve en fysieke functie is één van de drie onderdelen van het model. In deze populatie was de cognitie relatief goed omdat de meeste mannen een hoge score behaalden bij de 'Mini-Mental State Examination'. Met betrekking tot de beoordeling van het fysieke functionele vermogen suggereren we dat

het voldoende zou kunnen zijn om de fysieke prestatie middels de methode van Guralnik en de spierkracht te meten.

Het vermijden van ziekte en handicap is het tweede deel van het model van Rowe. In deze populatie hadden mannen met bepaalde ziekten een lager fysiek vermogen, zowel subjectief als objectief gemeten. Dit toont deels de correctheid van het model aan. Tevens suggereren we dat (subklinische) atherosclerose ook een aspect van dit deel van het model is.

Ten slotte, het behoud van een actieve betrokkenheid bij het leven is het derde deel van het model van Rowe. Fysieke prestatie en eenzaamheid waren negatief aan elkaar gerelateerd, wat de overlap tussen 'fysiek functioneel vermogen' en het 'betrokkenheid met het leven' deels aantoont. Bovendien hadden mannen die eenzaam waren een mindere kwaliteit van leven.

De meeste aspecten van de verschillende onderdelen van het model waren gerelateerd met kwaliteit van leven en overleving of mortaliteit. Dit toont het gecombineerde deel van de drie onderdelen van het model aan.

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